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Regulation of Seed Consumption in the Obligate Pollination – Seed Predation Mutualism Between a Yucca and a Yucca Moth

by

Judith Miriam Shapiro

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

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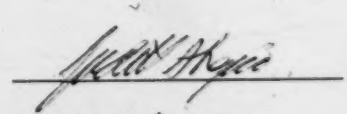

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Abstract

Yucca kanabensis and *Tegeticula altiplanella* have an obligate pollination – seed predation mutualism. This mutualism is based on reciprocal exploitation, and I addressed the question of what limits exploitation of the yucca seed resource by yucca moth larvae. First, I tested and rejected the hypothesis that moth egg mortality resulting from yuccas' selective abscission of flowers with heavy egg loads is density-dependent, and therefore potentially population regulating. Second, I found that there was highly density-dependent egg mortality in fruit. As moths oviposit into flowers, they damage some ovules. As more moths oviposit into a flower, more ovules are damaged, and a higher proportion of eggs are adjacent to a damaged ovule. Eggs that are adjacent to damaged ovules do not survive. Density-dependent *Tegeticula* egg mortality resulting from different causes may be a common mechanism limiting *Yucca* seed consumption costs and maintaining the balanced exploitation that is the yucca – yucca moth mutualism.

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Chapter 1

Introduction

1.0 Summary

Mutualism encompasses a diverse group of interspecific interactions that are beneficial to both partners. A common theme in the literature on mutualism is its potential instability, which may lead to a shift from mutualism to antagonism (e.g. parasitism) or non-interaction, and potentially to the extinction of both partners, if the interaction is unregulated (Axelrod and Hamilton 1981, Addicott 1981, Dean 1983, Keeler 1985, Soberon and Martinez del Rio 1985, Bull and Rice 1991, Bronstein 1994a, Pellmyr and Huth 1994, Doebeli and Knowlton 1998, Herre *et al.* 1999). Several factors are predicted to destabilize mutualisms. One important destabilizing factor is the conflict of interests that usually occurs between partners. Most mutualisms are best described as mutual exploitation, rather than reciprocal altruism (Janzen 1983, Bronstein 1994b, Herre *et al.* 1999) and, often, each organism would increase its fitness by increasing its exploitation of the other. Such conflicts of interest are particularly important in the obligate pollination/seed predation mutualism between yuccas and yucca moths. Determining what factors influence the costs and benefits experienced by mutualists is central to understanding the ecological and evolutionary function of mutualisms (Herre *et al.* 1999).

I investigated two mechanisms that might limit the level of *Yucca kanabensis* seed predation by *Tegeticula altiplanella* larvae. The first mechanism is egg mortality arising from yuccas' selective abscission of flowers with heavy egg loads, which might be density-dependent and help to regulate moth density. Selective abscission is commonly seen as the primary mechanism limiting seed predation costs incurred by yuccas (Pellmyr and Huth 1994, Bronstein 2001). The second mechanism I investigated is egg mortality in retained, developing fruit, which might directly limit seed consumption within fruit as well as help to regulate moth density. Egg mortality occurs in fruit of many yucca species (Riley 1892, Addicott 1986, Keeley *et al.* 1984,1986, Addicott and Bao 1999), but has not received sufficient attention as a potential mechanism for limiting exploitation of yuccas by their pollinator/seed predator, yucca moths.

1.1 What is mutualism?

Mutualism is widely understood to be an interaction from which both partners benefit. More specifically, when the lifetime reproductive success of individuals in each of two or more species increases as a result of their interaction, the species are mutualists (Addicott 1995). This contrasts with antagonistic interactions such as predation, parasitism, and competition. It also contrasts with commensalism, or facilitation, interactions from which one partner benefits and by which the other is not affected.

Mutualism applies to a wide variety of interspecific interactions. They can be obligate (necessary for survival and/or reproduction) or facultative (not necessary for survival or reproduction) for one or both species involved. Mutualisms may be obligate in some habitats and facultative in others. The types of benefits accrued in mutualistic interactions vary tremendously (e.g. pollination, habitat provision, nutrient transfer). Benefits may be direct (e.g. pollination) or indirect, meaning that they depend on the presence of a 3rd species (e.g. negatively affecting a competitor or predator, or positively affecting a mutualist). Mutualism may be symbiotic, meaning that one organism lives in or on the other (Starr 1975) or, at the opposite extreme, involve no physical contact (e.g. plants with a shared pollinator that attract disproportionately more pollinators in locales where plants are sympatric). Mutualism refers to an ecological interaction and does not define the evolutionary history of the interaction; many mutualisms have, in fact, evolved from parasitic interactions (including the yucca – yucca moth mutualism, Pellmyr and Thompson 1992). Organisms may be highly coevolved with their current mutualist partners or not at all coevolved. Interactions may be consistently mutualistic or they may be conditional, with the outcome depending on biotic or abiotic conditions (Bronstein 1994b).

As well as being diverse, mutualism is also ubiquitous in nature (Herre *et al.* 1999). The commonness of mutualism is not surprising because different species typically have different skills or efficiencies; by taking advantage of one another's capabilities, each stands to gain a net benefit. For instance, insects are mobile and are therefore much better adapted to transport pollen from flower to flower than are plants, whilst plants are autotrophs, meaning that they can convert energy from the sun into food, which insects can not do. Thus mutualisms can be thought of as "biological markets" in

which species exchange commodities or services that are relatively inexpensive for them to produce in exchange for commodities or services that are expensive or impossible for them to produce (Bronstein 2001). Because mutualism involves the exchange of products or services, both partners typically incur costs as well as benefits, with the costs being outweighed by the benefits. When one organism's benefit is the cost to its mutualistic partner, a conflict of interest may arise with each partner being selected for increased exploitation of the other.

1.2 Why might mutualisms be unstable and what might stabilize them?

A common theme in the literature on mutualism is the potential instability of mutualistic interactions (Axelrod and Hamilton 1981, Addicott 1981, Dean 1983, Keeler 1985, Soberon and Martinez del Rio 1985, Bull and Rice 1991, Bronstein 1994a, Pellmyr and Huth 1994, Doebeli and Knowlton 1998, Herre *et al.* 1999). Two types of instability are increased vulnerability to extinction of mutualistic species (Dodd and Linhart 1994, Pellmyr *et al.* 1996a) and risk of shifting from mutualism to an antagonistic interaction (Keeler 1985, Bronstein 1994b, Holland and Fleming 1999, Bultman *et al.* 2000) or to non-interaction (Keeler 1985). Three purported reasons for the instability of mutualism that I will briefly discuss are increased dependence on a particular species for survival (in obligate mutualism), conditionality, and conflicts of interest. Conflicts of interest present particularly interesting challenges to mutualism, particularly in the mutualism between yuccas and yucca moths. The presence of cheater species external to the interaction that exploit mutualisms is a fifth source of instability, but I will not address that issue. For a discussion of the effects and regulation of cheater species, see Soberon and Martinez del Rio (1985), Yu (2001), and Bronstein (2001), and see James (1998) for mechanisms that prevent excessive exploitation of yuccas and yucca moths by cheater moth species.

1.2.1. Dependence on a species for survival (obligate mutualisms)

The dependence of one species on another for survival and/or reproduction can be a source of instability in obligate mutualisms (Pellmyr *et al.* 1997). Simply put, if one species becomes extinct, so will its obligate mutualist. To be clear, this issue relates to the advantages and risks of specialization vs. generalization, not mutualism vs. antagonism

per se: A parasite that is highly host specific (as many are) also depends on its host species' survival for its own existence. The mutualism between yuccas and yucca moths is obligate for both partners and as such each could be seriously affected by declines in the other's density. I describe in section 1.3 some factors that reduce each partner's vulnerability to fluctuations in the other's density.

1.2.2. Conditionality

The occurrence, strength, and outcome of species interactions often depend on the abiotic and biotic context, i.e. the conditions within which interactions occurs (Abrams 1987, Cushman and Addicott 1991, Bronstein 1994b). Important sources of conditionality include the presence of a third species, the availability of external resources, and the density of partners (as mentioned above).

For example, ants and aphids have a mutualistic interaction in which ants consume the energy-rich excretions (honeydew) of the phloem-feeding aphids, and the aphids are protected from predation. However, if ants are given unlimited access to a honey solution, they may switch from tending aphids to eating them (Offenberg 2001). Another example of conditionality exists in the interaction between mycorrhizal fungi and plants. Most vascular plants have symbiotic associations between fungi and their roots. These associations are called mycorrhizae and are generally considered to be mutually beneficial (Raven *et al.* 1999). The mycorrhizal fungi increase their host plants' ability to capture water and nutrients such as phosphorus. The fungal partner, in exchange, receives carbohydrates and vitamins from the host plant (Raven *et al.* 1999). The "classic" mycorrhizal mutualism is usually mutualistic, but ranges from parasitic (on the plant) to neutral to mutualistic (Johnson *et al.* 1997). The plant only receives a net benefit from the exchange if the resources gained from the interaction are in limiting supply, and the magnitude of the benefits decreases as plant density increases (Johnson *et al.* 1997).

The commonness of conditional outcomes in interactions raises the question of what is truly meant by the term "instability" of mutualism and what it means for a mutualism to "break down". Many researchers describe the vulnerability of mutualisms to shifting to antagonistic interactions such as parasitism (Keeler 1985, Bronstein 1994a,

Holland and Fleming 1999, Bultman *et al.* 2000). The idea that a mutualism "breaks down" if it becomes parasitic does not adequately describe conditional interactions whose outcomes regularly vary between parasitic and mutualistic. It does not seem useful to consider the mutualism to be "breaking down" each time the interaction shifts to parasitism. In contrast, an interaction that is consistently mutualistic could potentially become consistently parasitic if one partner evolves a novel capacity that allows it to more fully exploit its partner. This would seem to qualify as dissolution of the mutualism. Humphries (1998) has suggested that conditionality may be capable of destabilizing mutualism. It is perhaps not the conditionality itself so much as the delicately balanced conflicts of interest, which are often revealed by conditionality, that destabilize mutualisms. Conflicts of interest and their resolution are discussed in the following section.

1.2.3. Conflicts of interest

Although mutualistic interactions are mutually beneficial to participating species, the participating species' interests are not necessarily perfectly aligned. In fact, given that conflicts of interest can even occur within the genomes of individuals when genetic elements compete for transmission opportunity (Werren *et al.* 1988, Hurst *et al.* 1996), it is hardly surprising that conflicts of interest would arise in interspecific interactions. In a mutualism, one organism generally exchanges a product or service for a different product or service with its partner. Sometimes the organism provides a product or service that is a byproduct of its own metabolism and incurs no cost from this, such as aphids that produce "honeydew" excretions which ants harvest. Usually, however, the organism incurs both costs and benefits from the interaction. Natural selection should favour the maximization of fitness benefits and minimization of fitness costs in each partner, which often means favouring increased exploitation. Since each partner is under selection for increased exploitation of the other, some models suggest that mutualisms are "evolutionarily unstable", and that the evolution of "cheating" by one partner may lead to the extinction of both partners (Pellmyr *et al.* 1996a). Alternatively, selection for increased exploitation might lead not to the extinction of the species, but to a shift of the

mutualism into an exploitative interaction (e.g. parasitism, predation, herbivory) (Keeler 1985, Bronstein 1994a, Holland and Fleming 1999, Bultman *et al.* 2000).

Since conflicts of interest are the norm in mutualistic interactions and yet mutualism is ubiquitous in nature, a fundamental question in the study of mutualisms is what prevents the dissolution of the mutualisms into antagonistic interactions, and perhaps even the extinction of partner species? Mutualisms are best seen as mutual exploitations that confer net benefits on both species (Janzen 1983, Bronstein 1994a, Herre *et al.* 1999), but what factors limit the level of exploitation that occurs?

There have been some attempts to address this question for particular interactions (e.g., Pellmyr and Huth 1994, Addicott and Bao 1999, Bultman *et al.* 2000, Holland and DeAngelis 2001, Jaeger *et al.* 2001), but there is not a satisfying universal framework for addressing it (Herre *et al.* 1999). Perhaps there cannot be, given the enormous diversity of mutualistic interactions. However, several mechanisms that may facilitate the maintenance of mutualisms are listed below. These mechanisms may result from coevolved adaptations, preadaptations, or from constraints external to either organism. They are not mutually exclusive; multiple modes of regulation may occur in any given interaction (Addicott and Bao 1999).

- Partner fidelity (Axelrod and Hamilton 1981, Clay 1990, Ewald 1993)
  Repeated interactions between individuals who can recognize one another and recall the outcome of previous interactions may promote cooperation. Vertical transmission can be considered an extreme form of partner fidelity in which a symbiont's offspring are transmitted to its host's offspring, and thus the symbiont's transmission opportunities depend on the host's reproductive success.
- Partner choice and retaliation against individuals who do not cooperate (Bull and Rice 1991, Stephenson 1981, Denison 2000)
  If organisms can choose their mutualist partners or defect against those who are non-cooperative, they can ensure they receive net benefits from their partners. Organisms might filter out individuals who are non-cooperative or species that are non-mutualists.
- Tight linkage of costs and benefits, so that benefits cannot be accrued without incurring costs (Addicott 1995, Anstett *et al.* 1997, Anstett 2001)

Having benefits tightly linked to costs prevents an organism from cheating, i.e. taking the benefits of the interaction and not reciprocating. Figs, for example, are pollinated by seed predators. After feeding on fig ovaries as larvae, adult female wasps emerge from figs carrying pollen with them to pollinate flowers in the figs they will lay their eggs in. Figs thus directly depend on the fig wasps emerging from their fruit for the male component of their reproductive success and they cannot gain that benefit without incurring the cost of some seed predation. In contrast, fig wasps do not have any individual advantage in not laying eggs in all female flowers, so some other mechanism(s) must limit larval consumption of seeds in figs.

- Mortality that acts at the appropriate life stage of a mutualist, particularly density-dependent mortality (e.g. Fleming and Holland 1998, Bultman *et al.* 2000, Jaeger *et al.* 2001).

  This mortality, whether externally induced or partner-induced, can prevent the mutualist from becoming numerous enough to overexploit its partner. For example, in a mutualism between a fly and a fungus, the fly "pollinates" the fungus by transferring fungal spermatia among fungi while searching for oviposition sites (Bultman *et al.* 2000). Fly larvae consume ascospores, the products of cross-fertilization, but eggs experience high rates of mortality that increase as egg numbers on host fungi increase. This egg mortality limited exploitation of the fungus by larvae, resulting in a net benefit to the host fungus from its "pollinating parasite" in each of the three years of Bultman *et al*'s study (2000).

- Regulation of investment into mutualism, making resources or services available only when the benefits of mutualism are high (e.g. Agrawal and Fordyce 2000, Heil *et al.* 2001)

  For example, many plants use indirect defences against herbivory. Indirect defence, also called biotic defence, involves the utilization of another party such as ants to provide protection in exchange for rewards such as nectar, food bodies, or shelter. Lycaenid butterfly larvae also produce nectar rewards for ants, and the ants protect the larvae from predators and parasitoids. Sometimes the host's

indirect defence is induced; that is, in response to detection of herbivory or the presence of predators, the plant or larva increases reward production to attract protectors. The advantage of induced defences over constitutive defences is that the costs associated with production of rewards are only incurred when the benefits of the mutualism are high.

1.3 Yuccas and Yucca Moths

Yuccas (*Yucca* genus, Agavaceae) and yucca moths (*Tegeticula* genus, Prodoxidae) have an obligate pollination – seed predation mutualism; yuccas are only pollinated by yucca moths, and yucca moths oviposit in yucca flowers. Developing seeds are the sole food source for yucca moth larvae. In spite of obvious conflicts of interest, the yucca – yucca moth interaction is estimated to have persisted as a mutualism for forty million years (Pellmyr and Leebens-Mack 1999). Because of the apparent stability of the mutualism in spite of obvious conflicts of interest, and because it is a relatively simple one to one mutualism, the yucca – yucca moth interaction is useful for investigating mechanisms that promote stability in mutualism.

Yuccas are long-lived polycarpic plants and, except in rare instances (Dodd and Linhart 1994), their only pollinator is the yucca moth (Riley 1892, James *et al.* 1993). During their short adult lifespans (generally less than five days, Riley 1892, Dodd and Linhart 1994) yucca moths mate in flowers, female moths collect pollen from anthers, then pollinate and oviposit in one and two day old flowers. Yucca moths do not oviposit anywhere other than yucca flowers. Pollen collection and pollination by yucca moths is "active", with maxillary appendages and behaviours existing entirely for that purpose (Pellmyr 1997). The result of active pollination is that pollen does not limit the availability of developing seeds, the sole food source for the pollinator's larvae. Thus, neither species can reproduce sexually without the other. The benefit to the yucca is seeds fertilized and the primary cost to the yucca is seeds eaten.

Such extreme specialization, resulting in both the yucca and the yucca moth depending entirely on one another for sexual reproduction, would seem to increase both species' risk of extinction (see section 1.2.1). Several factors mitigate this risk. Yuccas are long-lived and reproduce clonally as well as sexually. If their pollinator moths

become locally extinct for several seasons, yuccas will survive until the immigration of pollinators from surrounding areas. Moths would seem to be more vulnerable because if the population emerges out of synchrony with the yuccas for one season, or if the yuccas do not set fruit due to extreme weather conditions for one season, that cohort of moths dies without reproducing. However, two factors alleviate this risk. First, moths diapause for varying numbers of winters (Riley 1892, Fuller 1990), so if one summer there is no successful reproduction, there will be others from the same cohort emerging over the next several seasons. Yucca moths may even be capable of diapause for well over a decade, as individuals of the related moth *Prodoxus y-inversus* have emerged from diapause in lab conditions after as many as 19 years, the longest reported insect diapause (Powell 1989). Secondly, the moths emerge and the yuccas flower over a period of a month or more, so some moths will emerge out of synchrony with the yuccas during any given season, and some will emerge when flowers are receptive. During my second field season, early emerging moths did not have reproductive success because a late frost killed the early flowering yuccas. However, the later flowering yuccas and later emerging moths were not harmed by the frost.

Additionally, there may be sufficient variation in traits such as self-compatibility and stigma morphology that yuccas can adapt in response to extended pollinator shortages, rather than losing all sexual reproduction. This evolutionary "adaptability" is indicated by *Yucca glauca* being self-incompatible where it is not pollen-limited in New Mexico (Fuller 1990) and being self-compatible where it is pollen-limited at the elevational and latitudinal edges of its range (Dodd and Linhart 1994, Donna Hurlburt personal communication). The stigmatic surface in yucca flowers is generally recessed in the style so that deliberate pollen tamping is needed to effect pollination (Riley 1892, Rau 1945). However, Dodd and Linhart (1994) found that the pollen-limited yuccas at high elevation sites in Colorado received some pollination from a fly as well as yucca moths, and that these yuccas' flowers could be successfully pollinated by simply placing pollen on their stigmas, without any tamping down. Although the evolutionary history of these populations is not known, the observed variation indicates that while we may consider self-incompatible, *Tegeticula*-dependent yuccas to be obligate mutualists ecologically,

self-compatibility and style morphology appear to have the potential to adapt in the face of extended pollinator shortages.

Yucca moths' benefit from the mutualism is seeds as a food source for their offspring. The conflict of interests in this interaction is clear; yuccas would benefit in the short term by minimizing seed predation, and yucca moths would benefit by maximizing use of the seed resource. Unlike figs, whose pollen dispersal is effected by the larvae produced in syconia (and thus benefits are tightly linked to costs), an individual yucca does not depend directly on the pollinator eggs in its flowers for any element of its fitness. Larvae that emerge from the fruit overwinter in the soil for one or more winters and emerge in the spring as adults. Since they obtain no direct fitness gain from the moth larvae that emerge from their fruit, yuccas that can kill all of the eggs in their fruit will eliminate the seed predation cost associated with the mutualism. In fact, at least one yucca species, *Y. baccata*, does have individuals who "cheat", and no larvae survive in the fruit of these "cheater" individuals (Bao and Addicott 1998). "Cheater" individuals have inviable ovules in the apical region of their fruit, where moths oviposit, and the eggs in this region invariably perish. However, cheater flowers have fewer viable ovules (fewer potential progeny) than cooperator flowers with viable ovules in the apical regions. The cost to the "cheater" of losing those apical ovules as potential progeny is outweighed by eliminating seed consumption when moths are abundant. However, when moths are rare, as they may become if cheating increases in frequency, "cheater" yuccas are at a selective disadvantage relative to "cooperator" yuccas who have viable ovules in the apical region of their fruit. Frequency-dependent selection is thus expected to prevent cheating from reducing the moth population to extinction (Nathan Forsyth, personal communication). "Stability" in this case may involve cyclical dynamics, with periods of pollinator abundance and great success of "cheater" yuccas relative to "cooperator" yuccas, followed by periods of reduced pollinator abundance and increased "cooperator" success relative to "cheater" success (Forsyth, personal communication).

A yucca moth may reduce her costs if she does not pollinate each flower in which she oviposits, and certainly such "cheating" is observed (Tyre and Addicott 1993), but since yucca moths are the only yucca pollinators such facultative cheating will be limited. If several moths oviposit in a flower and none pollinate it, all of their eggs will perish in

the abscised flower. Addicott and Tyre (1995) have found that yucca moths are more likely to pollinate flowers that have not already been visited when they encounter them. In this way a moth may save some pollen and some time, but the flower is still likely to have been pollinated. If, instead, she pollinated every flower she visited on an inflorescence, the moth might need to move between inflorescences more often, perhaps increasing the cost of time spent searching for yuccas and the risk of being eaten while in flight.

Yucca moths might be expected to maximize the benefits they gain from the interaction by utilizing the seed resource as efficiently as possible. If moths consumed the entire seed resource, yuccas would have no reproductive success. However, since yuccas live for decades and also reproduce clonally, the negative feedback on moths of a decline in their mutualist partner would be in the distant future relative to moth generation time. This negative feedback could not select against overexploitation of the seeds. Despite this, seed predation is quite low; only 15% of yucca seeds, on average, are consumed by moth larvae (Addicott 1986) and complete seed destruction in a fruit is very rare (Humphries 1998). It is not clear what limits the level of seed predation by larvae.

The interaction between yuccas and yucca moths occurs on multiple levels, and mechanisms occurring at different levels likely interact with one another. Each flower has six locules, and ovipositions occur in a specific location of each locule. One might look for regulating mechanisms at the locule level, the flower/fruit level, the inflorescence level, and the plant population level, both within and between seasons. In this thesis I focus on two mechanisms that might limit the seed predation costs yuccas incur, one operating at the inflorescence level and the other at the locule level. One mechanism that may reduce yuccas' seed consumption costs is called selective abscission. Fruit production in most yuccas is limited by resources other than pollen, such as water, and yuccas can only retain approximately 10% of their flowers (Addicott 1986). This presents an opportunity for yuccas to mature their highest quality fruit, those with the best pollen quality and quantity and the fewest moth eggs. Pellmyr and Huth (1994) have suggested that selective abscission of flowers with many eggs in them helps to regulate the level of seed predation experienced by yuccas. A second mechanism that may reduce yuccas' seed consumption costs is egg mortality in retained fruit. Addicott and Bao (1999) have

recently presented some evidence for density-dependent egg mortality within locules of developing fruit. In this thesis I consider the role that both selective abscission and egg mortality have in determining the level of seed predation experienced by *Y. kanabensis*.

In Chapter 2 I examine egg mortality that is created by yuccas' selective abscission of flowers with many eggs. *Yucca kanabensis* has been shown to be resource-limited (vs. pollen-limited) and can only retain approximately 10% of flowers. The abscission of extra flowers has also been shown to be selective: Flowers with more eggs are more likely to be abscised than flowers with fewer eggs (Addicott and Bao 1999). Eggs in abscised flowers invariably perish. The primary question I address in Chapter 2 is whether egg mortality resulting from selective abscission is density-dependent. As yucca moths become more numerous relative to yuccas, the average number of eggs laid per flower increases, and thus the potential for increased seed destruction may increase. If this source of egg mortality is density-dependent, it may help to regulate moth populations, preventing them from becoming numerous enough to overexploit the yucca seeds.

Several authors have suggested that there may be egg mortality in developing fruit of several yucca species (Keeley *et al.* 1984, 1986, Addicott 1986). Addicott and Bao (1999) showed that there is significant egg mortality in *Y. kanabensis* fruit. In Chapter 3 I examine *T. altiplanella* egg mortality in retained, developing *Y. kanabensis* fruit. How much egg mortality takes place in fruit? Is it density-dependent? What causes this mortality? Does egg mortality play a large role in limiting seed destruction within fruit?

One facet of the interaction between *Y. kanabensis* and *T. altiplanella* that I hypothesize influences both of the above mechanisms, selective abscission and egg mortality in fruit, is ovule damage resulting from oviposition. *Tegeticula altiplanella* females have long ovipositors that permit them to oviposit right into the locular cavity of a flower (Pellmyr 1999). Oviposition by this moth damages some ovules, which do not develop into seeds (Addicott and Bao 1999). Since moths oviposit in a stereotyped fashion in a specific location within the locule, multiple moths ovipositing into a locule create a contiguous row of damaged ovules, a "damage zone". In Chapter 2 I ask whether the number of damaged ovules in a flower is a cue for selective abscission and, if it is, how closely the number of damaged ovules reflects the number of eggs laid in a flower.

In Chapter 3 I ask if eggs inside the damage zone of a locule have a different survival rate from eggs outside the damage zone of a locule.

Finally, I will consider the roles of selective abscission and egg mortality relative to one another in determining the seed predation costs that yuccas experience. If yuccas selectively abscise flowers with large damage zones, and eggs experience higher mortality inside damage zones, do yuccas actually retain flowers with more surviving larvae? How density-dependent is the egg mortality in fruit? Do flowers with more eggs laid in them have significantly more larvae feeding in them, and if they do not, what is the role of selective abscission in regulating yuccas' costs?

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Chapter 2

The Effect of Selective Abscission by *Yucca kanabensis* on *Tegeticula altiplanella* Population Dynamics: a Density-dependent Mortality Factor?

2.0 Introduction

Mutualisms are relationships between two or more species from which each gain a net benefit. Sometimes described as reciprocal altruism (Trivers 1971), mutualism can also be usefully thought of as balanced mutual exploitation (Janzen 1983, Bronstein 1994, Herre *et al.* 1999). The latter description emphasizes the disruptive potential of conflicts of interest between partner organisms. In most mutualistic interactions the partners' interests are not perfectly aligned; an increase in the net benefit to one partner entails a decrease in the net benefit to the other. The existence of conflicts of interest leads to the question of why mutualism is so common, and in many cases, so stable (Axelrod and Hamilton 1981, Bull and Rice 1991, Bao and Addicott 1998, Doebli and Knowlton 1998, Herre *et al.* 1999). What prevents one species from overexploiting the other?

In the many mutualistic interactions in which there exist clear conflicts of interest but in which both partners still gain a net benefit, what limits exploitation? The optimality approach to studying adaptation assumes that individual selection will result in the best possible phenotype *subject to constraints* (Alcock 1993). Organisms are not "perfectly adapted" because they face trade-offs and because natural selection is limited to acting on traits currently in existence in a population. Therefore, although each mutualist may appear to be under selective pressure to increase its exploitation of the other, trade-offs or constraints that are internal or external to the interaction may limit exploitation, maintaining the mutual net benefit. The goal of this study was to examine a mechanism that might limit exploitation of *Yucca kanabensis* (Agavaceae) by its obligate mutualist, *Tegeticula altiplanella* (Prodoxidae).

Yucca kanabensis and *T. altiplanella* have an obligate pollination – seed predation mutualism. In the spring, after mating, a gravid female moth will collect pollen from a yucca flower, and will fly to another yucca. There, she will oviposit an egg into the locule of a fresh flower and then actively pollinate that flower using her specialized maxillary tentacles. A moth may oviposit multiple times in a flower and she may visit many flowers during her short adult life. Multiple moths commonly visit any given flower. When a fruit

develops, the larvae will eat some of the developing seeds, chew their way out of the fruit, drop down to the soil, and overwinter in a pre-pupal diapause.

On average, 12% of viable seeds in *Y. kanabensis* fruit are consumed by larvae (Addicott 1986). It is rare for all of the seeds in a yucca fruit to be consumed by larvae (Rau 1945, Humphries 1998). In the absence of any trade-offs or constraints, one would expect natural selection to favour more efficient exploitation of the seed resource by *T. altiplanella*. What constraints act to limit seed predation? One possibility is that there is density-dependent mortality of *T. altiplanella* preventing the moths from reaching densities that would allow the overexploitation of yuccas. This study addresses the specific question of whether a particular mechanism, known as selective abscission, creates density-dependent moth egg mortality. A model of the interaction between *Y. kanabensis* and *T. altiplanella* predicts that egg mortality resulting from selective abscission will be density-dependent (Addicott unpublished).

Fruit production in yuccas is limited by resources other than pollen ("resource-limited"), and yuccas are only able to develop approximately 10% of flowers into fruit (Addicott 1986). The rest of the flowers are abscised (dropped) by the plant and the eggs in those flowers invariably perish, as do the ovules. Egg mortality that results from resource-limited abscission is density-dependent for a limited range of moth densities. As visitation (the proportion of flowers on a yucca with at least one oviposition) increases, resource-limited abscission (the proportion of visited flowers that are abscised) necessarily increases (Figure 2.1). For example, an inflorescence that has 100 flowers of which 10 have been visited will not abscise any of the visited flowers and no moth eggs will by killed by abscission. An inflorescence with 100 flowers, all of which have been visited, will abscise 90% of the visited flowers on average. Therefore, on average, 90% of the moth eggs on that inflorescence will perish in abscised flowers. As moth density increases, the number of moths that visit a yucca increases, and the proportion of flowers on its inflorescence that are visited ("visitation") increases (Figure 2.2). As visitation increases, egg mortality due to resource-limited abscission necessarily increases (Figure 2.1). This creates density-dependent mortality. However, visitation can not increase beyond 100%. Once all of the flowers on yuccas are visited by moths, further increases in moth density will yield higher numbers of eggs per flower, but resource-limited

abscission will still only kill 90% of the eggs, on average. If 10% of flowers are randomly retained, 90% of moth eggs, on average, will die due to resource-limited abscission, regardless of the number of moth eggs laid in flowers. Thus, egg mortality due to resource-limited abscission ceases to be density-dependent at high moth densities. At some sites visitation varies between 50 and 80%, with resource-limitation creating density-dependent moth mortality (Addicott 1998). *Tegeticula altiplanella* densities are known to be consistently high in other locations, with yuccas typically having over 95% of their flowers visited (Addicott pers. com., personal observation). Resource-limited abscission mortality can not be density-dependent at these sites.

However, egg densities are not the same in all flowers on a plant. Selective abscission of yucca flowers with many yucca moth eggs is a mechanism whereby yuccas can potentially reduce the number of larvae in their fruit and thereby reduce the level of seed mortality they experience. Selective abscission was predicted to occur by Addicott (1986) and by Bull and Rice (1991), and has been demonstrated in *Y. filamentosa* (Pellmyr and Huth 1994), *Y. whipplei* (Richter and Weis 1995), and in *Y. kanabensis* (Addicott and Bao 1999).

If an inflorescence has 100 flowers, 90 of them are visited, and 9 of them are retained, the mortality of eggs in the abscised flowers can be broken into two categories: egg mortality due to resource-limited abscission, which is the mortality rate that would occur if abscission had been random, and egg mortality due to selective abscission, which is the mortality that occurs above and beyond resource-limited abscission, as a result of the selectiveness of abscission. At sites with high moth density, where all of the flowers on yuccas are visited (such as the sites I used in my experiment), resource-limited abscission does not create density-dependent egg mortality. The primary question I address in this chapter is, does selective abscission create density-dependent mortality for the *T. altiplanella* population at these sites?

If yuccas always abscised flowers with more than a certain number of eggs in them, selective abscission would result in strongly density-dependent, even overcompensating, moth mortality. With a fixed threshold, as moth density increased, the number of eggs in retained fruit would not increase (Addicott and Bao 1999). Yuccas whose flowers all had more eggs in them than the threshold number would not retain any

fruit. However, Addicott and Bao (1999) state that field observations of yuccas show that such a threshold, if it exists, is too high to be an important regulating factor. The threshold number of eggs above which all flowers are abscised by a yucca appears to be highly contingent on the flowers available to it. Yuccas do not reduce the proportion of fruit they retain in response to heavy egg loads (personal observation). It appears that the yucca's strategy is more along the lines of "retain as many flowers as possible, given resource limitation, and select those flowers with as few eggs as possible."

This strategy is illustrated in a simulation model developed by Addicott (unpublished). His model predicts that if yuccas always retain 10% of flowers and always choose the best flowers (i.e., those with the fewest ovipositions), then egg mortality due to selective abscission will be density-dependent. However, yuccas do not retain precisely the 10% of flowers with the fewest ovipositions, in part because other factors such as pollen amount and quality and position on the inflorescence also affect flower quality (Humphries 1998). Additionally, yuccas abscise flowers before eggs hatch and before larval feeding begins. Since eggs have not hatched yet, it is likely that yuccas make the selective abscission decision based on an indirect and probably imperfect cue for egg number. Such factors might affect the potential density-dependence of egg mortality due to selective abscission. This study addresses the question of whether yuccas, with their limited selective abscission capacity, generate density-dependent egg mortality.

Due to temporal, spatial, and stochastic variation, and possibly due to differences in attractiveness to moths, each inflorescence is visited by a different number of moths. Generally, inflorescences that are visited by more moths have more ovipositions in their flowers, because moths do not avoid ovipositing in previously visited flowers (Figure 2.3). Therefore I address this question at the inflorescence level: I ask if egg mortality resulting from selective abscission is positively correlated with the mean number of eggs per flower on a yucca (Figure 2.4). If yuccas that are visited by more moths kill eggs at a higher rate than yuccas that are visited by fewer moths, then presumably populations and seasons with higher overall numbers of moths per inflorescence have higher selective abscission-induced moth mortality rates. However, the relationship between moth density and selective abscission mortality will be complicated by variation in inflorescence

density. The proportion of yucca rosettes that produce an inflorescence varies from year to year and during seasons with higher inflorescence density there is less moth egg mortality resulting from abscission (Figure 2 in Addicott 1998). Despite the variation in inflorescence density, if there is a density-dependent relationship between the average number of ovipositions per flower on an inflorescence and egg mortality generated by selective abscission, it will potentially help to regulate moth density.

In a second experiment I address the question of whether damage moths do to ovules during oviposition triggers selective abscission. Oviposition damage may be a cue for selective abscission of fertilized flowers since ovules that are damaged during oviposition apparently die before the selective abscission "decision" takes place (Pellmyr and Leebens-Mack 2000). If damage does trigger selective abscission, how accurately does the number of damaged ovules in a flower reflect the number of eggs in it? Do flowers with more eggs, on average, have more damaged ovules across the entire range of ovipositions? Or, does the number of damaged ovules reach an asymptote after some number of ovipositions, preventing differentiation of flowers with more than that number of eggs? If flowers with more than a given number of eggs cannot be differentiated based on damage zone, this would limit yuccas' selective abscission and the moth mortality it creates.

2.1 Methods

2.1.1 Study Site and Organisms

I studied the interaction between *Yucca kanabensis* McKelvey (Agavaceae) and its pollinator / seed predator moth *Tegeticula altiplanella* Pellmyr (Prodoxidae). The taxonomic status of yucca populations near Kanab is unresolved (McKelvey 1947, Webber 1953, Cronquist *et al.* 1977) and, following Addicott (1998), I use McKelvey's (1947) classification, calling the yuccas between Kanab and Zion National Park *Y. kanabensis*.

My field experiments took place over two field seasons at four sites near Kanab, Utah. Sites were selected because they only had one pollinator moth, *T. altiplanella*, and so the interaction was not complicated by the presence of a second pollinator species. During the first field season, yuccas flowered between June 13th and July 15th, 1999. The second field season was May 23rd to July 7th, 2000. The "Yellowjacket" site is located on the road to Coral Pink Sand Dunes State Park (37° 7' 30" N, 112° 40' 45" W, 1800m elevation) near Yellowjacket Ranch. Two sites were located along Hancock Road, in a Wildlife Study Area adjacent to Coral Pink Sand Dunes State Reserve; "Hancock1" is located at the top of the Hancock Road hill (37° 2' 19" N, 112° 42' 54" W) and "Hancock2" is located approximately two kilometres northeast of "Hancock1", about one hundred metres northeast of the campground (37° 2' 5" N, 112° 44' 0" W). "Red Knoll" is at the base of Red Knoll, on its eastern side, about 15 kilometres northwest of Kanab (37° 9' 12" N, 112° 38' 0" W).

Yucca kanabensis is a common plant at all sites, alongside *Juniperus monosperma* Engelmann, *Artemesia tridentata* Nuttall, *Wyethia scabra* Nuttall, and scattered clumps of *Quercus gambellii* Nuttall. All study sites are located on open, sandy ridges. *Yucca kanabensis* produces a raceme of approximately 50 – 150 flowers in 1 – 3 waves with acropetalous flowering (flowers opening in order from base to apex) within each overlapping wave. Yucca flowers are hermaphroditic, and the stigmas are generally receptive at the same time as anthers dehisce. Some flowers are protogynous; the stigmas are receptive and the pollen unavailable during the first night after anthesis, and the stigmas are receptive and anthers dehisced on the second night (personal observation).

Flowers open in early evening and remain receptive to pollen for two nights. After this time, petals begin to wilt and the ovary either begins to shrivel and then abscises, usually within five days, or shows enlargement. Only 5 – 10% of flowers mature into fruit (Addicott 1985, 1998). Fruit reach full size within thirty days and final seed maturation takes another thirty days (Humphries 1998). When the fruit dries and dehisces, it releases seeds that are thin and largely wind-dispersed. As well as reproducing by seed, *Y. kanabensis* reproduces vegetatively, resulting in clones of up to 44 rosettes (Addicott and Tyre 1995). They are long-lived polycarpic perennials and do not produce an inflorescence every year.

Tegeticula altiplanella is one of two yucca moth species that pollinate *Y. kanabensis*, but it is the only one that occurs at the sites I used. *Tegeticula altiplanella*, also referred to as "deeps", oviposit through the carpel wall into the locular cavity, hence their common name. In contrast, *T. superficiella* ("shallows") have shorter ovipositors and oviposit into the carpel wall, damaging no ovules. Oviposition by *T. altiplanella* damages some ovules, which never develop into seeds (Addicott and Bao 1999). *Tegeticula altiplanella*'s oviposition behaviour is highly stereotyped; all oviposit about 1/3 of the way down the long axis of the ovary from the apical end, creating a contiguous row of damaged ovules which I will refer to as the "damage zone" (Figure 2.5). This is visible as a constriction as the fruit grows. Larvae hatch from their eggs after 9-11 days (James 1998, personal observation) and during their second instar begin consuming some of the developing seeds. During their fourth and final instar, larvae chew out of the fruit, drop to the ground and burrow 5 – 20 cm into the sand (Fuller 1990). Yucca moth larvae diapause for one to several winters in a cocoon and pupation occurs several weeks before adults emerge in the spring (Fuller 1990).

2.1.2 Experiment 1- Density-dependence of Selective Abscission – Induced *Tegeticula altiplanella* Mortality

This experiment addresses the question "is the egg mortality that results from selective abscission higher in inflorescences with a higher average number of eggs per flower?" Answering this required counting the number of eggs in all of the abscised flowers and retained fruit of inflorescences. I tried to capture as much natural variation

(spatial and temporal) in moth densities on inflorescences as possible as well as doing some experimental manipulation of moth densities on inflorescences to better assess the density-dependence of moth mortality.

2.1.2.1 Field Methodology

I selected inflorescences that had not begun flowering and labelled the buds on the inflorescences according to their axil position and flowering wave using a Sharpie® Fine Point Permanent Marker. Every morning I checked each inflorescence to record which flowers had opened, which flowers had abscised, and to collect abscised flowers. Abscised flowers were stored in 70% ethanol for later dissection. I re-labelled flowers as the ovaries enlarged and the petals withered to ensure that I could always identify and age a fruit.

I put cages made of green garden fencing around some inflorescences to deter browsing by free ranging domestic cattle. The cages allowed moths to pass through to the flowers. However, the cages increased the wind resistance of inflorescences, so I only used them in times and places where cattle were known to be active. Once all of the flowers on an inflorescence had been open for two nights, and had completed their interaction with adult moths, I put a light mesh bag made of window screening on the inflorescence to protect it from cattle. Mesh bags did not make inflorescences more vulnerable to wind damage, but they exclude moths and could not be used on younger inflorescences. Many inflorescences were eaten by cattle despite my precautions[1]. In addition, one inflorescence was damaged by a mouse and one was damaged by an ant colony. I did not use any data from partially consumed inflorescences. Initial and final sample sizes are included in Table 2.1. It is interesting, and perplexing, to note that moth densities appear to be higher at sites where a high proportion of inflorescences are eaten

[1] Near the end of my 2nd field season, I received advice from Paul Chapman at the Bureau of Land Management in Kanab to put long pieces of red flagging tape on inflorescences, because cows are apparently uncomfortable around something red flapping in the wind (and the wind is ever-present). I did not use this technique long enough to confirm its reliability, but future researchers may wish to try it.

by cattle each summer than at sites that do not have cattle on them and that have only a small proportion of inflorescences eaten by deer (Addicott pers. comm.).

I harvested fruit when I was certain they were not going to be abscised. In 1999 I harvested fruit from an infructescence when all of its fruit were 15 days old and older and there had been no abscission for two or more days. This harvesting protocol was conservative, with a low probability of counting fruit that were going to abscise as retained, since over 95% of flowers that abscise do so by day 12 (Humphries 1998, James 1998). However, I found that as fruit age, it becomes difficult to count ovipositions in them. Therefore, when harvesting retained fruit, there is a trade-off: As fruit age, they are less likely to be abscised by the plant (and therefore the error of considering an abscised fruit as retained decreases), but oviposition holes also become stretched as fruit grow (and therefore error in oviposition counts increases). In order to be more confident in my oviposition counts and still be confident that harvested fruit were being retained, I changed the harvesting protocol in 2000 to harvest fruit at 13 days. Ovipositions are still clear in 13 day old fruit, and my data from 1999 confirmed that well over 95% of flowers that were going to abscise did so by day 12 (Figure 2.6).

In order to capture as much natural variation as possible in mean floral egg load per inflorescence, I utilized inflorescences from the entire flowering season and from several sites. As well, I attempted to increase the variation experimentally on some plants by manipulating moth density on them:

Protocol to reduce moth visitation:

I flushed moths out of the flowers of these inflorescences every other evening before moth activity began and put a cage made of window screening on the inflorescence to exclude moths. Since flowers are receptive to moths for two nights, this meant each flower would be exposed to moths for half of its receptive period, reducing the average number of ovipositions in flowers.

Protocol to increase moth visitation:

I caged inflorescences every other evening and stocked them with a large number of moths collected from non-experimental plants. I placed about three female moths per open flower into the cage with the inflorescence, as well as leaving moths that were resting in the inflorescence's flowers. This should have more than doubled normal levels

of oviposition per flower (Wilson and Addicott 1998). Some of the moths that were transferred to the inflorescence already carried pollen in their maxillary tentacles from other inflorescences, but by keeping moths caged in all evening, I increased the chance that moths would pollinate flowers with pollen from the same inflorescence. However, the inflorescences were uncaged every other night, during which time they would receive natural levels of cross-pollination. Unfortunately, this technique proved to be labour intensive in 1999 and so using it reduced the sample size. Thus, there was a trade-off between attempting to increase variation in visitation by putting cages with many moths on inflorescences and by increasing sample size. Since the caging did not substantially increase moth visitation, I decided in 2000 that a larger range of visitation would be achieved by having a larger sample of inflorescences instead of using this technique.

2.1.2.2 Dissection Methodology

Abscised flowers and retained fruit were either dissected within one day of being collected or harvested, respectively, or were preserved in 70% ethanol (ETOH) for later dissection. I estimated the number of eggs laid in abscised flowers and retained fruit by counting oviposition tracks using a dissecting microscope at 10x magnification. Addicott (unpublished data) has found a 1:1 relationship between *T. altiplanella* ovipositions and eggs in fresh *Y. kanabensis* flowers. To count oviposition tracks, thin sections of the carpel wall were sliced away with a scalpel, revealing the tracks (Figure 2.7). Blue food dye diluted in water and dabbed on to the freshly sliced carpel assisted my counting by pooling up in the tracks and making them more visible.

Some flowers and fruit were dissected during the flowering season, and many were preserved in 70% ethanol (ETOH) and dissected later in Edmonton, Alberta. I did not find any difference in clarity of oviposition marks between fresh and preserved flowers.

2.1.2.3 Analysis

I calculated the *k* value ("killing power") of selective abscission for each inflorescence and I also calculated the average number of eggs per flower for each inflorescence (which is correlated with moth density relative to inflorescence density,

Figure 2.3). I plotted *k* values for inflorescences against their mean egg loads in order to determine if, and over what range, moth egg mortality due to selective abscission was density-dependent. *K* values are a standard measure of the intensity of mortality between one life-history stage and another, or the mortality caused by a particular factor (Varley *et al.* 1975). The *k* value of stage x is calculated as:

$K\ x = \log_{10}(N_x / N_{x+1})$ where,

N_x = the number of individuals alive at the beginning of stage x and,

N_{x+1} = the number of individuals alive at the beginning of the following stage.

The *k* value for selective abscission measures the mortality of moth eggs caused by selective abscission, the mortality that occurs over and above that resulting from resource-limited abscission. Each inflorescence is visited by a different number of moths, which is reflected by a different mean number of eggs per flower. If moth egg mortality due to selective abscission is density-dependent, the *k* value for an inflorescence will be positively correlated with its mean number of eggs per flower (top line, Figure 2.4). Overall, this should mean that sites and years with higher moth density relative to inflorescence density will have higher moth egg mortality rates due to selective abscission. Inflorescences will generally have more eggs per flower and higher *k* values. However, as mentioned in Section 2.0, the relationship between moth density and selective abscission mortality rate will be complicated by variation from year to year in inflorescence density.

I calculated *k* values for selective abscission by substituting the number of pollinator eggs that would be in the retained fruit of a yucca if abscission were random for N_x and the number of eggs that were actually in the retained fruit of a yucca (as estimated by the number of oviposition marks) for N_{x+1} in the formula above. The number of eggs that would be in the retained fruit of a yucca if abscission were random is estimated by multiplying retention (the proportion of visited flowers retained) by the total number of ovipositions in all of the flowers. A *k* value for selective abscission is calculated for each inflorescence. Thus, the *k* value for selective abscission is:

K for selective abscission = $\log_{10}$(number of eggs that would be in fruit if retention was random / number of eggs actually in fruit)

which equals:

K for selective abscission = $\log_{10}$(retention x number of eggs in all visited flowers / number of eggs actually in fruit).

The larger the difference between the egg load in the average visited flower and the egg load in the average retained fruit, the larger the k value. I will use "selective abscission mortality" as shorthand for *T. altiplanella* egg mortality that results from selective abscission, and the k value for selective abscission is the measure of that mortality.

Moths do not show any avoidance of one another, or avoidance of flowers that already have ovipositions in them (Wilson and Addicott 1998) so as the number of moths that visit an inflorescence increases, the average number of eggs per flower also increases (Figure 2.3). Therefore, the average number of eggs in an inflorescence's flowers serves as a proxy for the number of moths that visited it.

2.1.2.4 Simulations

After conducting this field experiment and analyzing the results, I did a simulation exercise to help interpret the results. I created "hypothetical inflorescences" by randomly generating numbers of ovipositions per flower using a normal distribution. I used the normal distribution because 41 / 49 of my inflorescences had frequency distributions of numbers of eggs per flower that did not significantly deviate from normality ($p>0.05$, K-S test in SPSS). For each "hypothetical inflorescence" I calculated the mean number of eggs per flower, the standard deviation of eggs per flower, and the maximum possible k value for selective abscission. I used this simulation exercise to lend insight into what parameters create the potential for density-dependent selective abscission mortality and what parameters prevent the possibility of density-dependence. The simulations are explained in the Discussion of this chapter.

2.1.3 Experiment 2- Damage Zone as a Cue for Abscission

This experiment addressed the questions "does oviposition damage to ovules trigger selective abscission and, if it does, how well does the number of damaged ovules

in a flower reflect the number of ovipositions in it?" To answer these questions, I counted the ovipositions and damaged ovules in abscised flowers and retained fruit.

2.1.3.1 Field Methodology

Using Wilson and Addicott's methods (1998), I removed 80% of the buds on inflorescences so that each remaining flower had approximately a 50% chance of being retained. Using plants with only 20% of their flowers is more efficient for detecting differences between abscised flowers and retained fruit, since an inflorescence with 100% of its flowers is only expected to retain approximately 10% of them[2]. Removing most of the flowers/buds from an inflorescence seems very intrusive but it apparently does not reduce retention rates by yuccas as long as the plant is left with at least the 10% that it would have originally retained (Humphries 1998).

I also hand-pollinated every flower with one anther load of cross pollen (see 2.1.3.2 below) to minimize variation in flower quality due to pollen load, and to therefore more efficiently examine the effects of oviposition number and number of damaged ovules on flower retention.

I selected 25 inflorescences at Red Knoll (12 survived), 14 inflorescences at Yellowjacket (9 survived), and 6 inflorescences at Hancock2 (5 survived). I removed 80% of the buds on the inflorescences and labelled the remaining buds with a Sharpie® Fine Point Permanent Marker. Every morning I checked each inflorescence to record which flowers had opened the night before, hand-pollinated those flowers, and collected any abscised flowers. I previously found that it is very difficult to determine the number of damaged ovules in an abscised flower once it falls off the plant. By this point, all of the ovules in the flower are completely withered and damaged ovules cannot be

[2] In hindsight, I realize that visitation (the proportion of flowers on an inflorescence that are visited) rates are much higher at my sites than at sites used by Wilson and Addicott. Higher visitation means that retention rates- the proportion of visited flowers retained- are lower than Wilson and Addicott's 10%. Thus, I could have increased the efficiency of this experiment further by removing 85% of flowers from each inflorescence.

differentiated from undamaged ovules. However, there are signs that a flower is going to be abscised before it actually falls; a white line forms at the receptacle of the flower and the pistil begins to decrease in size and shrivel instead of grow. For this experiment, therefore, I watched flowers carefully and collected abscising flowers before they abscised so that I would be able to count damaged ovules. I harvested fruit at 13 days of age, considering them to be retained (see Figure 2.6).

2.1.3.2 Hand Pollination Protocol

I selected pollen donor inflorescences that were at least 25 m away from experimental plants in order to ensure outcrossing. I removed any open flowers and put a mesh cage made of window screening over the inflorescences to exclude moths. Every morning I collected all of the newly opened flowers from pollen donors and placed them in a cooler to use for hand pollination of experimental plants that morning. To pollinate a newly opened flower on an experimental plant, I used a yucca blade leaf to detach the pollen mass from an anther of a pollen donor flower, and gently placed the pollen down the recipient flower's style. I transferred all of the pollen on an anther, which is more than enough to ensure full fertilization of ovules (Humphries and Addicott 2000). Every experimental flower received fresh cross pollen from a flower that had opened the night before.

2.1.3.3 Dissection Methodology

I estimated the number of eggs laid in locules by counting oviposition tracks (see 2.1.2.2). I sliced down to expose the ovary and, removing ovules one by one with forceps, I noted the number and locations of ovules damaged by oviposition.

Analyses are based on 26 inflorescences. Twelve inflorescences were from Red Knoll, 9 from Yellowjacket, and 5 from Hancock2.

2.1.3.4 Analysis

I used SPSS to perform logistic regressions on flower fate (retained vs. abscised), using the number of ovipositions in a flower and the number of damaged ovules in a flower as covariates. The purpose was to determine which covariate better predicted

retention, and to determine if both covariates (which are positively correlated with one another) are significant when they are both included in the regression model.

Secondly, I plotted the number of damaged ovules against the number of ovipositions in a flower to gauge the correlation between the two and to determine if the number of damaged ovules reaches an asymptote as it increases with the number of ovipositions. If ovule damage asymptotes with oviposition number, flowers with more than the threshold number of ovipositions could not be distinguished from one another on the basis of damage zone size.

2.2 Results

2.2.1 Experiment 1- Density-dependence of Selective Abscission – Induced *Tegeticula altiplanella* Mortality

There was no evidence to suggest density-dependent moth mortality resulting from selective abscission. The *k* value for each inflorescence is shown with its mean number of eggs per flower in Figure 2.8. The linear term in the regression is negligible and is not statistically significant (b=0.001, t=1.15, p=0.25); only the constant is significant (constant=0.137, t=3.65, p<0.001). This means that the *k* value for selective abscission is significantly different from zero (i.e. that selective abscission of flowers with many eggs did occur) across the entire range, but that it is not affected by the mean number of eggs in an inflorescence's flowers. This density-independence occurs within each site; the slope of a first order regression of *k* values on average number of eggs per visited flower is negligible and statistically insignificant within each site (between 0.001 and 0.004, p>0.05 within each site). Therefore, the pattern is not driven by the results at a particular site or during only one of the seasons. Egg mortality resulting from selective abscission was no higher on inflorescences with 70 eggs per flower than on inflorescences with 10 eggs per flower. This means that egg mortality due to selective abscission was not shown to be density-dependent at these sites during these seasons.

Inflorescences with higher variation in egg number per flower do not have higher *k* values, indicating that selective abscission is not limited by the natural levels of variation (Figure 2.9).

As well as calculating *k* values for each inflorescence, I calculated the maximum possible *k* value, the egg mortality that would have occurred if the inflorescence had retained the flowers with the fewest eggs. *K* values for all inflorescences were below their maximum possible *k* value (Figure 2.10), suggesting that either the cue for abscission is imperfect, that factors other than egg number also affect flower quality, or both.

The most surprising result in this experiment is that the maximum *k* values for the inflorescences were also not density-dependent (Figure 2.11). Therefore, even if these plants did have the ability to determine egg loads of flowers precisely and even if all other variables affecting flower quality (e.g., pollen source and amount, flower position,

timing of flower presentation) were equal, selective abscission mortality would not have been density-dependent on these inflorescences. This result is contrary to the findings of Addicott's model (unpublished).

2.2.2 Experiment 2- Damage Zone as a Cue for Abscission

2.2.2.1 Is the number of damaged ovules a cue for selective abscission?

Both the number of ovipositions and the number of damaged ovules in a flower are significant factors in the probability of retention in separate logistic regressions ($p<0.001$). However, ovipositions create damaged ovules and the number of damaged ovules in a flower is correlated with the number of ovipositions (see below). When both the number of ovipositions and the number of damaged ovules in a flower are included as covariates in a logistic regression on retention, only the number of damaged ovules is significant ($p=0.002$) (Table 2.2). When the number of damaged ovules in a flower is known, also knowing the number of ovipositions in that flower does not significantly increase one's ability to predict that flower's fate ($p=0.26$). Since damage to ovules is a better predictor of retention than the number of ovipositions is, it is reasonable to hypothesize that damage might be a factor that influences retention "decisions" by the plant. Ovipositions probably influence flower retention primarily or entirely through their damage to ovules. Essentially, flowers with more damage are less likely to be retained (Figure 2.12).

Note, however, that flowers with the fewest damaged ovules were not the most likely to be retained (Figure 2.12). This pattern indicates that flowers that were visited by the fewest moths were not the most likely to be retained, because they received less pollen than flowers visited by slightly more moths. I hand-pollinated each flower with a full anther load of cross-pollen, which reduced the effect that pollen had on retention, but clearly did not eliminate it. This may be because flowers that were visited by many moths had cross-pollen from multiple sources, and flowers that were visited by few moths had primarily cross-pollen from one source. *Yucca filamentosa* has been shown to retain flowers with pollen multiple sources over flowers with pollen from a single source (Huth and Pellmyr 2000). However, the minor effect that pollen had on retention decisions did

not affect my ability to determine the relative roles of oviposition number and oviposition-damaged ovules in retention (Table 2.2).

In the above analysis, flowers from different plants were pooled together, potentially masking a 'plant effect'. The result of the multiple logistic regression would be spurious if, first, some plants were more susceptible to oviposition damage than others and, second, if the susceptible plants retained fewer fruit (proportionally) than less susceptible plants. In this scenario, even if damage zone is not a cue for abscission, the number of damaged ovules in a flower will be appear to be a significant predictor of its fate in my analysis. Indeed, there was a significant difference between plants in the relationship between oviposition number and number of damaged ovules in their flowers (ANCOVA, plant: $F=5.32$, d.f.=27, $p<0.001$). However, there was no relationship between the average number of damaged ovules per oviposition in a plant's flowers and the proportion of flowers it retains (linear regression, $F=0.15$, d.f.= 26, $p=0.70$, adjusted $r^2=0.03$). This rules out the alternative scenario that would create a spurious result.

2.2.2.2 How accurately does the number of damaged ovules reflect the number of eggs?

The relationship between ovipositions and damage zone in locules of abscised and retained flowers is shown in Figure 2.13. Each locule is oviposited in and damaged independently, and so evaluating the pattern of oviposition damage on ovules is most straightforward at the locule level. Number of damaged ovules increases with number of ovipositions across the entire observed range of oviposition numbers, but there is a statistically significant decline in the rate of increase (negative 2nd order term: $p<0.001$). The levelling off observed is not complete in the observed range of ovipositions; the number of damaged ovules does continue to increase with oviposition, and so within the observed range locules with more ovipositions have, on average, more damaged ovules. There appears to be more variation in number of damaged ovules as oviposition number increases (Figure 2.13), but that is a product of smaller sample sizes rather than increased natural variability, and does not necessarily indicate a decreasing quality of signal. Given that yuccas selectively abscise flowers with large damage zones, it is not surprising that the number of damaged ovules is significantly higher for a given number of ovipositions in locules of abscised flowers than in retained fruit (Figure 2.13).

Although each locule is oviposited in separately, each locule with its own "damage zone", the plant must retain or abscise an entire flower (with its six locules). The relationship between oviposition number and number of damaged ovules in flowers is shown in Figure 2.14. Again, over the observed range of ovipositions, the amount of ovule damage increases at a slightly declining rate. Only samples greater than five are plotted in Figure 2.14, and one can see that by eliminating tiny samples, the variation in damage zone size does not increase with number of ovipositions (unlike in Figure 2.13, where all samples are plotted). On average, flowers with more ovipositions have more damaged ovules, and so the damage zone cue is useful across the observed range of ovipositions.

2.3 Discussion

One way that the seed predation costs incurred by yuccas might be limited, preventing the yucca – yucca moth mutualism from shifting to parasitism, would be for moth density to be regulated. Selective abscission receives much attention for its potential to "regulate" the yucca – yucca moth mutualism (Pellmyr and Huth 1994, Bronstein 2001). Eggs in abscised flowers inevitably perish and this mortality can be broken into two categories: resource-limited abscission mortality and selective abscission mortality. Resource-limited abscission mortality is very high, because yuccas only retain approximately 10% of flowers (Addicott 1986). However, at sites with high moth density, such as the ones I studied, resource-limited abscission egg mortality is not density-dependent. Visitation at these sites is consistently close to 1.0 and resource-limited abscission mortality is approximately 90%, regardless of changes in moth density. In the absence of any other regulating mechanism, the average number of eggs in retained fruit could potentially increase indefinitely as moth density increased.

Addicott (unpublished) has developed a simulation model which suggests that moth egg mortality resulting from selective abscission ("selective abscission mortality") might be density-dependent, except for at very high moth densities[3]. Addicott's model predicts that as moth density increases, k values for selective abscission will increase until moth densities are extremely high, at which point k values will plateau and then decline. The reason for the predicted density-dependence is that inflorescences in his model that experience higher moth density have higher average numbers of eggs per flower, but they always have a small number of flowers with very few ovipositions. Thus, if yuccas retain the 10% of flowers with the fewest eggs, then the number of eggs in retained fruit of yuccas won't change much as moth density increases, but the average number of eggs per visited flower will increase rapidly. This means the difference

[3] Holland and DeAngelis (2000) have also developed a model which predicts that selective abscission can regulate pollinator populations, but their model's assumptions do not fit the yucca – yucca moth mutualism in fundamental ways, and so I will not refer to their model in this discussion.

between the average number of eggs per flower on a yucca and the average number of eggs per retained fruit will increase dramatically as moth density increases. The increasing difference between average egg load in retained fruit and average egg load in all visited flowers results in density-dependent selective abscission mortality rate (as measured by *k* values). As moth density becomes high, there are fewer flowers that "escape" with few eggs laid in them, and *k* values plateau. When all flowers have so many eggs in them they can't be distinguished from one another, *k* values drop to 0 (*k* of 0 means random abscission, no egg mortality created by selective abscission).

This was the first study to measure *T. altiplanella* egg mortality due to selective abscission at sites without another pollinator moth complicating results and with wide variation in moth densities experienced by yuccas (Experiment 1). *Yucca kanabensis* does not retain the exact flowers with the fewest eggs in them and so, based on the predictions of Addicott's model, I initially assumed that if selective abscission mortality was density-independent, this would indicate that either there were other factors (such as pollen amount and quality) affecting flower quality in such a way as to prevent density-dependent egg mortality, or that the cues yuccas use for selective abscission do not accurately reflect the number of eggs in a flower.

I found that *T. altiplanella* mortality due to selective abscission was density-independent (Experiment 1, Figure 2.8). However, I was surprised to also discover that even if the inflorescences *had* retained the flowers with the fewest eggs in them, egg mortality would have remained density-independent (Figure 2.11). In other words, the reason that the observed selective abscission-induced *T. altiplanella* egg mortality was density-independent was not necessarily related to the ability of yuccas to precisely detect ovipositions or due to other factors affecting flower quality. Addicott's prediction that density-dependent selective abscission mortality would occur if yuccas retained the flowers with the fewest eggs did not hold for yuccas at the sites I used during the flowering seasons of 1999 and 2000.

2.3.1 Simulations of Inflorescences and Conditions Affecting Potential Density-Dependence

In order for selective abscission mortality to be density-dependent, inflorescences with higher mean egg loads must always have some flowers with very few eggs on them, flowers that largely escape the attention of moths due to temporal variation in moth density over a yucca's flowering period. These flowers are key to the potential for density-dependent selective abscission mortality. This means that the variation in number of eggs per flower on an inflorescence must increase with the mean number of eggs per flower on an inflorescence. I realized this simple truth through simulating yuccas by randomly generating egg numbers per flower using the normal distribution. First, I simulated inflorescences with a constant standard deviation of eggs per flower. In other words, all yuccas, regardless of the moth density they experience and the resultant mean egg loads in their flowers, had the same variation in number of eggs per flower. Simulations showed that if the standard deviation of number of eggs per flower is constant, then as the mean number of eggs per flower on an inflorescence increases, k values will decline, resulting in inversely density-dependent egg mortality (Figure 2.15). The reason for this result is that the difference between the mean number of eggs per flower and the mean number of eggs on the lowest 10% of flowers remains constant *in absolute terms* as the mean increases, but declines *proportionally* (Figure 2.16). Mortality rates are based on proportions, so the k value for selective abscission declines as well. I will hereafter refer to this as the "proportional effect".

For instance, suppose there are two yuccas that have different mean egg loads, but on which the variation in egg load is constant. A yucca experiencing low moth density has an average of 25 eggs per flower and its lowest flowers have an average of 11 eggs each. A yucca experiencing high moth density has an average of 50 eggs per flower and its lowest flowers have an average of 36 eggs each. The difference between the average flower and the average retained flower is 14 eggs in both cases, but that difference results in a higher rate of selective abscission egg mortality when there are fewer moths to begin with (the yucca with 25 eggs per flower). A mathematical explanation of this result, provided to me by Dr. Bertrand Clarke, is included in Appendix 2.1.

Thus, this first simulation made it is clear that variation in number of eggs per flower on inflorescences must increase with their mean number of eggs per flower for selective abscission mortality to be density-dependent. My next question was "how much does variation need to increase with mean number of eggs per flower for selective abscission mortality to be density-dependent?"

In my second simulation, the standard deviation increased linearly with mean egg load. For example, if a yucca with a mean of 25 eggs per flower had a standard deviation of 8, then a yucca with 50 eggs per flower had a standard deviation of 16. If the standard deviation of number of eggs per flower increases linearly with the mean number of eggs per flower on an inflorescence, then the difference between the smallest numbers of eggs on an inflorescence and the average number of eggs on an inflorescence will increase as the mean increases. Simulations showed that the increased difference between smallest egg numbers and average egg numbers cancels out the proportional effect (Figure 2.16). However the increasing variation only cancels out that proportional effect and does not create those flowers with very few eggs in them that are necessary for density-dependent selective abscission mortality. In simulations in which standard deviation increases linearly with mean egg load, egg mortality due to selective abscission was density-independent (results from model inflorescences with standard deviation = 0.30 times mean shown in Figure 2.17). Appendix 2.1 explains that regardless of the rate with which standard deviation increases as the mean number of eggs per flower increases, if the increase is linear, selective abscission mortality will be density-independent.

Finally, I simulated yuccas in which the standard deviation in eggs per flower increased quadratically (e.g., $0.01x^2$). I found that as mean egg load on inflorescences increased, the proportional effect (Figure 2.16) was overcompensated by the rapidly increasing difference between average egg load and smallest egg loads, and egg mortality due to selective abscission was density-dependent (Figure 2.19). In other words, this is the situation in which there are always enough flowers that escape with very few eggs in them (as in Addicott's model). The average number of eggs per retained fruit is not much higher in a yucca with a high density moth population than a yucca with a low density moth population.

2.3.2 Summary of Simulation Results

To summarize my simulation results, if the variation in number of eggs per flower was constant on yuccas experiencing different moth densities, selective abscission mortality was inversely density-dependent. Variation was not constant on my experimental inflorescences (Experiment 1) and selective abscission mortality was not inversely density-dependent (Figure 2.8). If the standard deviation increased linearly with mean number of eggs per flower on inflorescences, my simulations suggested that selective abscission mortality would be density-independent. The standard deviation of number of eggs per flower very clearly increased linearly with the mean number of eggs per flower on inflorescences in my field data (Figure 2.18) and selective abscission mortality was density-independent (Figure 2.8). In order for selective abscission mortality to possibly be density-dependent, as in Addicott's model, my simulations suggested that the standard deviation must increase quadratically with the mean number of eggs per flower. When this happens, inflorescences always have some flowers with very few eggs in them, and the difference between egg number in the average flower of an inflorescence and egg number in retained fruit increases sufficiently as mean egg load increases to overcome the proportional effect (Figure 2.16).

The fact that the standard deviation of eggs per flower on inflorescences I studied increased linearly with their mean number of eggs per flower is sufficient to explain the density-independence of k values. A quadratic increase of standard deviation with mean number of eggs per flower is a necessary (but not sufficient) requirement for density-dependent selective abscission-induced egg mortality. This requirement holds true at least for when eggs are normally distributed on inflorescences, and may change with significant departures from normality (e.g., a bimodal distribution).

2.3.3 Further Reasons For Density-Independence of Egg Mortality

My simulations suggest that standard deviation in egg load on inflorescences must increase quadratically with their mean number of eggs per flower for moth egg mortality due to selective abscission to be density-dependent. Standard deviation increased linearly with mean egg load in my field data (Figure 2.18), which simulations suggest is sufficient

to explain the observed density-independence of selective abscission egg mortality (Figure 2.8).

However, Figure 2.10 suggests that this is not the only reason for the observed density-independence. All of the inflorescences I studied had *k* values below the maximal *k* value, meaning that they were not retaining the flowers with exactly the fewest eggs. As the maximum possible *k* value on an inflorescence increased, the actual *k* value remained essentially constant (Figure 2.10). This lack of correlation between an inflorescence's actual *k* value and its maximum possible *k* value suggests that even if eggs were distributed differently on inflorescences such that the maximum possible *k* values were density-dependent (e.g., Figure 2.19), selective abscission might be limited by some other factor, preventing density-dependent egg mortality.

Yuccas with greater variation in flower egg loads have greater potential for selective abscission, and their maximum *k* values are higher. However, my yuccas' *k* values did not increase with variation in egg load (Figure 2.9) or their maximum *k* values (Figure 2.10). What limited a yucca's ability to respond to this greater variation with higher selectiveness? It appears that yuccas use the number of damaged ovules as a cue for abscission (Table 2.2., Millar 2001, Fuller 1990), which is not surprising, as evidence exists for other plants that damage to the seeds of very young fruit by insects can trigger abscission (Andersen 1988, Stephenson 1981 and references therein). The number of damaged ovules in a flower is correlated with the number of ovipositions in it (Figure 2.14). An imperfect correlation between the number of ovipositions and number of damaged ovules in flowers would result in *k* values below their maximum possible. Indeed, there is variation in the relationship between oviposition number and number of damaged ovules.

However, for the imperfection of the damage zone cue to explain why yuccas with greater variation in flower egg loads aren't more selective in their abscission than yuccas with less variation in egg loads, the damage zone cue must decrease in its usefulness at high egg loads. If the number of damaged ovules increases across a limited range with oviposition number, but asymptotes once a flower has more than X ovipositions, then yuccas would not be able to distinguish between flowers with more than the threshold number of ovipositions. As more and more flowers on an inflorescence

had greater than X ovipositions in them, the yucca's ability to be selective would decrease. One might expect such a levelling off of number of damaged ovules with oviposition, since moths all oviposit in the same location of a locule, but the damage zone does not level off within the oviposition range I observed (Figure 2.14).

The damage zone cue for abscission is not perfectly correlated with oviposition number in a flower, and therefore one expects the *k* value for selective abscission to be less than its maximum possible. However, the usefulness of the cue is not diminished at high egg loads, and so it should not affect the yucca's ability to respond to increased variation in its flower egg load. Yuccas with increased variation in their egg loads can potentially be more selective in their abscission, but they were not, and imprecision of the damage zone cue can not explain this result.

If the cue used for selective abscission, oviposition damage, does not prevent density-dependence of egg mortality, what does? An important factor in flower quality that was not included in this study is pollen amount and quality, and including this in a more comprehensive study might help explain why inflorescences' *k* values do not increase with their maximum possible *k* values.

Given that *Tegeticula* densities are observed to be relatively stable from year to year (Addicott 1998), something other than selective abscission must create density-dependent moth mortality, regulating the population size. In Chapter 3 I will demonstrate that it is moth mortality in retained fruit, rather than in abscised flowers, that is strongly density-dependent. Since the strongly density-dependent mortality takes place at the egg stage in fruit it regulates the moth population as well as preventing overexploitation of seeds within individual fruit.

2.4 Conclusion

This experiment found that *T. altiplanella* egg mortality due to selective abscission by *Y. kanabensis* was not density-dependent. The primary reason for density-independence was that the standard deviation of eggs per flower on inflorescences increased linearly with mean number of eggs per flower. At least for inflorescences with normal frequency distributions of eggs on flowers, the standard deviation of eggs per flower on an inflorescence must increase quadratically with mean number of eggs per flower in order for density-dependent selective abscission mortality to be possible. This is a necessary condition for density-dependent selective abscission mortality, but it is not sufficient on its own.

This is just one study and it is possible that in some circumstances the standard deviation of eggs per flower on inflorescences may increase quadratically with their mean. If standard deviation did increase quadratically with mean, there would also be other factors that might affect the density-dependence of egg mortality. Inflorescences in this study had k values that were independent of their maximum possible k values (Figure 2.10), indicating that even if standard deviation increased quadratically with mean, k values would still not be density-dependent. My data cannot explain why k values did not increase with the maximum possible k value. The number of damaged ovules in a flower, which appears to be a cue for abscission, increases with the number of ovipositions, but there is a significant amount of variation in this relationship. The imprecision of the damage zone – oviposition relationship would certainly reduce the egg mortality rate resulting from selective abscission. However, damage zone size does not asymptote within the observed range of oviposition numbers, so I would not expect the imprecision of the damage zone cue to affect the density-dependence of selective abscission egg mortality.

My results suggest that it is unlikely that egg mortality due to selective abscission is commonly density-dependent. At the sites I visited, *T. altiplanella* eggs are found in close to 100% of the flowers. With such high visitation rates, resource-limited abscission creates a large amount of egg mortality, but this mortality is not density-dependent. Something other than resource-limited and selective abscission mortality must keep the

moth population from increasing unbounded; Addicott (1998) has observed that moth populations appear to be relatively stable from year to year. Larval mortality has been noted in developing *Y. kanabensis* fruits (Addicott and Bao 1999), and so this source of moth mortality will be discussed in the following chapter.

Table 2.1. Sample sizes for Experiment 1, Density Dependence of Selective Abscission Moth Mortality

Year	Site	Treatment	Initial Sample Size	Number Inflorescences that survived
1999	Yellowjacket	None	21	10
	Yellowjacket	Moth density Reduced	6	3
	Yellowjacket	Moth density Increased	6	3
2000	Yellowjacket	None	12	11
	Hancock	None	41	12
	Hancock	Moth density Reduced	10	5
	Hancock 2	None	12	8
			Total Sample Size:	52

Table 2.2. Logistic regression of damage zone and ovipositions on flower fate. When both variables are included in model only damaged ovules is statistically significant. Overall model Chi-Square=39.22, df=1, $p<0.001$ n=354.

Variable	Coefficient	d.f.	Significance
Number of damaged ovules	-0.016	1	p=0.002
Number of ovipositions	-0.014	1	p=0.263
Constant	0.680	1	p=0.006

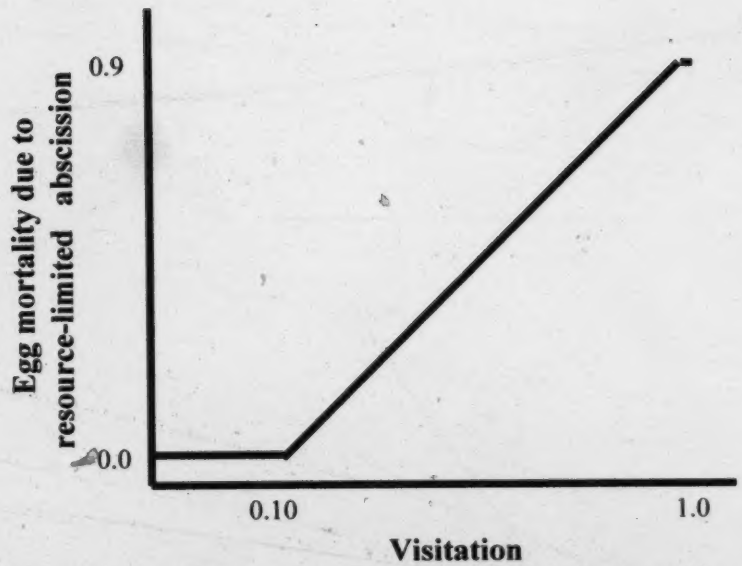

Figure 2.1. Egg mortality resulting from resource-limited abscission as a function of visitation (the proportion of flowers on an inflorescence that have at least one oviposition). Simplified schematic showing the direct consequence of resource limitation: as the proportion of flowers on a yucca that has been visited increases, the proportion of visited flowers that are abscised by the yucca increases. When yuccas have fewer than 0.10 flowers visited, they are pollen-limited and do not abscise any visited flowers. Therefore, no moth eggs die due to resource-limited abscission. When every single flower on a yucca has been visited, yuccas abscise 0.90 flowers on average (Addicott 1986). Therefore, on average, 90% of the moth eggs laid in the flowers of that yucca will perish due to abscission.

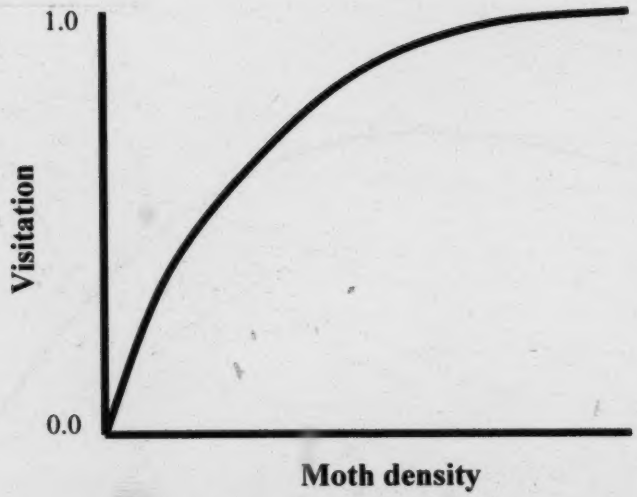

Figure 2.2. Schematic illustrating that as the moth density increases (relative to inflorescence density), visitation - the proportion of flowers on a yucca that are visited by moths- increases. The precise shape of the curve is not known. It is difficult to measure moth density in the field; and it changes over the flowering period of a yucca.

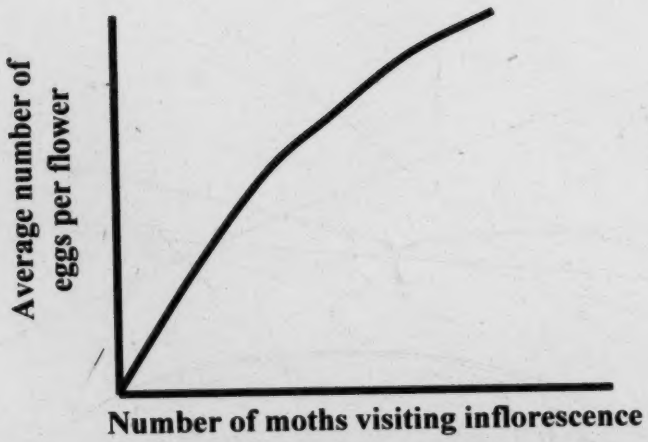

Figure 2.3. Average number of eggs per flower in a yucca as a function of number of moths visiting yucca (schematic). Once visitation (proportion of flowers visited) reaches 1.0, it ceases to be a useful indicator of the number of moths that visited a yucca. As moth density increases, visitation remains 1.0. However, the average number of eggs laid per flower continues to rise as more moths visit an inflorescence. Yucca moths do not appear to avoid ovipositing in previously visited flowers (Addicott and Tyre 1995, Wilson and Addicott 1998). Thus, the mean number of ovipositions per flower is the measurement I used as a correlate of the number of moths that visited an inflorescence (as per Addicott and Bao 1999).

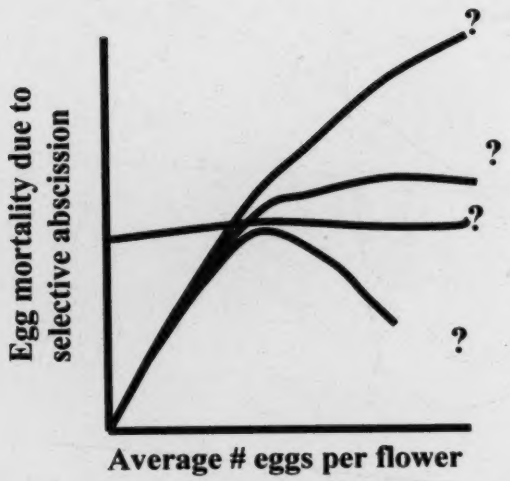

Figure 2.4. Possible outcomes of Experiment 1. The mean number of eggs per flower is used as a correlate of the number of moths that visited an inflorescence. Selective abscission mortality is the moth egg mortality due to the selectiveness of flower abscission, as measured by *k* value. The primary question of this study is whether selective abscission egg mortality is density dependent.

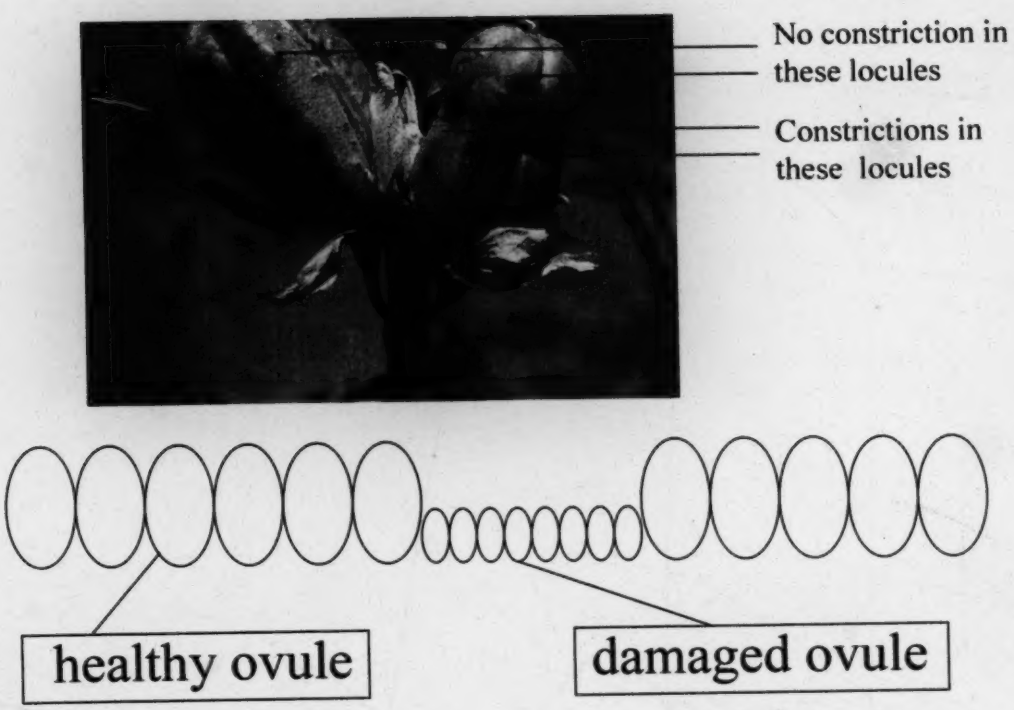

Figure2. 5. Damage Zone. Photo shows *Y. kanabensis* fruit with constricted locules, constrictions resulting from damage to ovules by *T. altiplanella* oviposition, and unconstricted locules (without ovipositions). Schematic diagram of one locule shows the "damage zone": a contiguous row of ovules that do not develop as a result of damage from oviposition. A damage zone is visually distinguishable 4 days after anthesis and results in a constriction as the fruit develops.

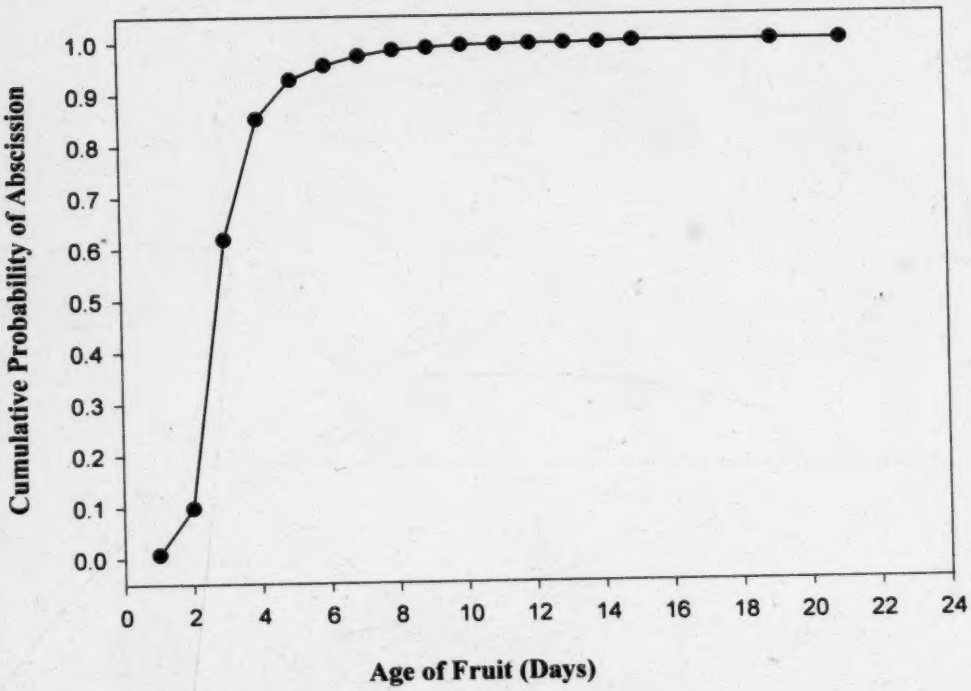

Figure 2.6. The cumulative probability that a flower will abscise by a given age. n = 1802 flowers.

Figure 2.7. Two oviposition holes visible in slice of carpel wall (10x). Blue colour is due to food dye, diluted in water, dabbed on to carpel wall to highlight oviposition tracks.

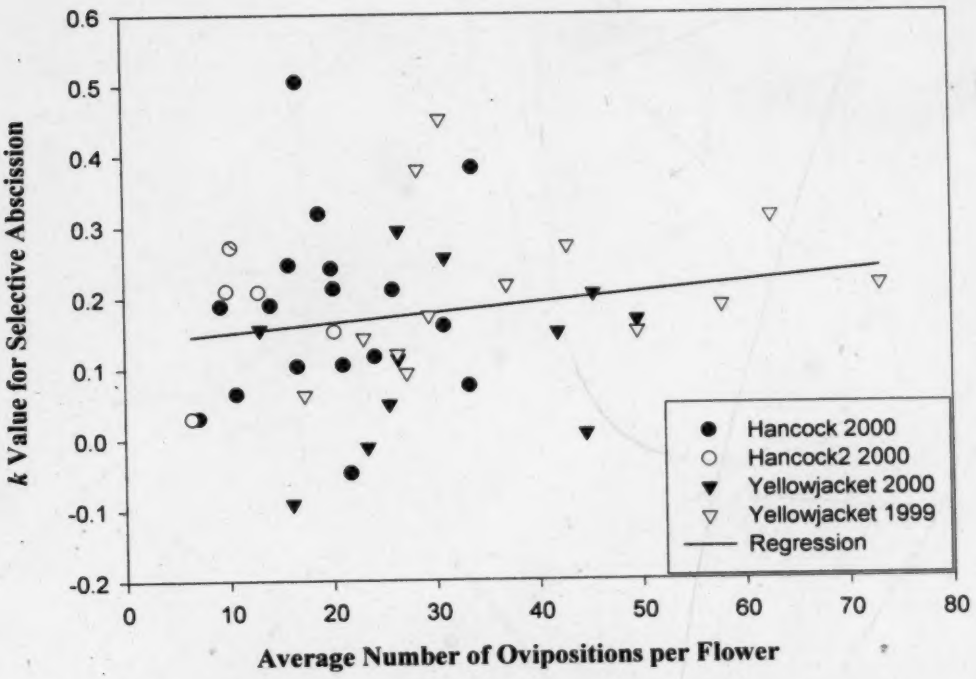

Figure 2.8. *K* value for selective abscission by an inflorescence plotted against the mean number of ovipositions per flower in that inflorescence. Each point represents one inflorescence (one outlier was removed), and the line represents the linear regression: $y = 0.137+0.001x$ ($r^2=0.029$, df=44). The legend shows which inflorescences are from which site and which year.

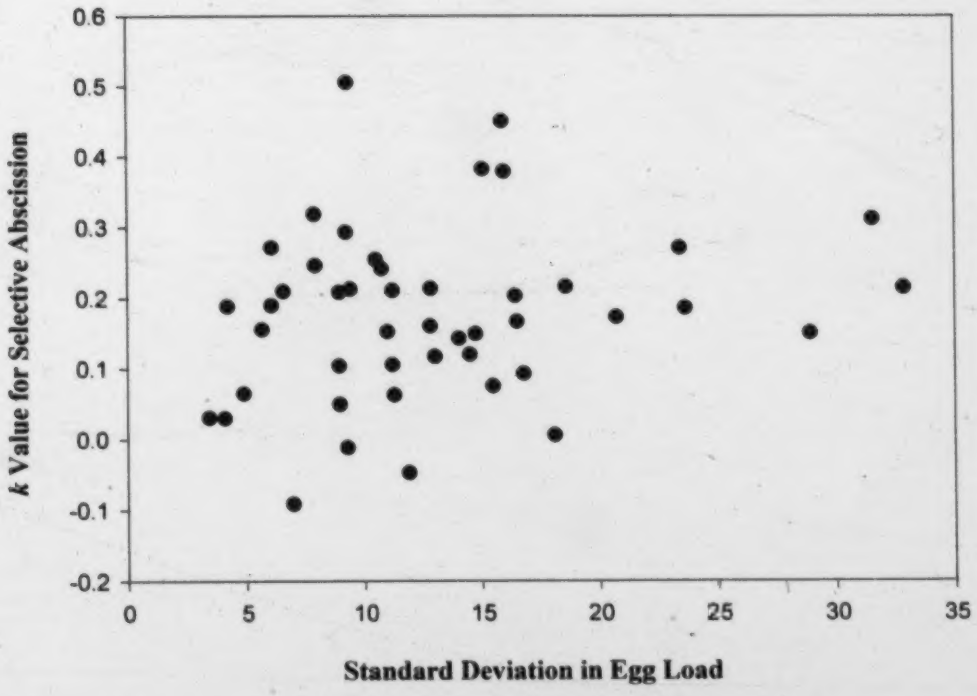

Figure 2. 9. Each inflorescence's *k* value plotted against the standard deviation of its oviposition numbers. Plants with higher variation in egg numbers per flower did not kill moths at a higher rate with selective abscission. Linear term in a regression has p = 0.187 (df=44). Same pattern is seen if coefficient of variation is used instead of standard deviation.

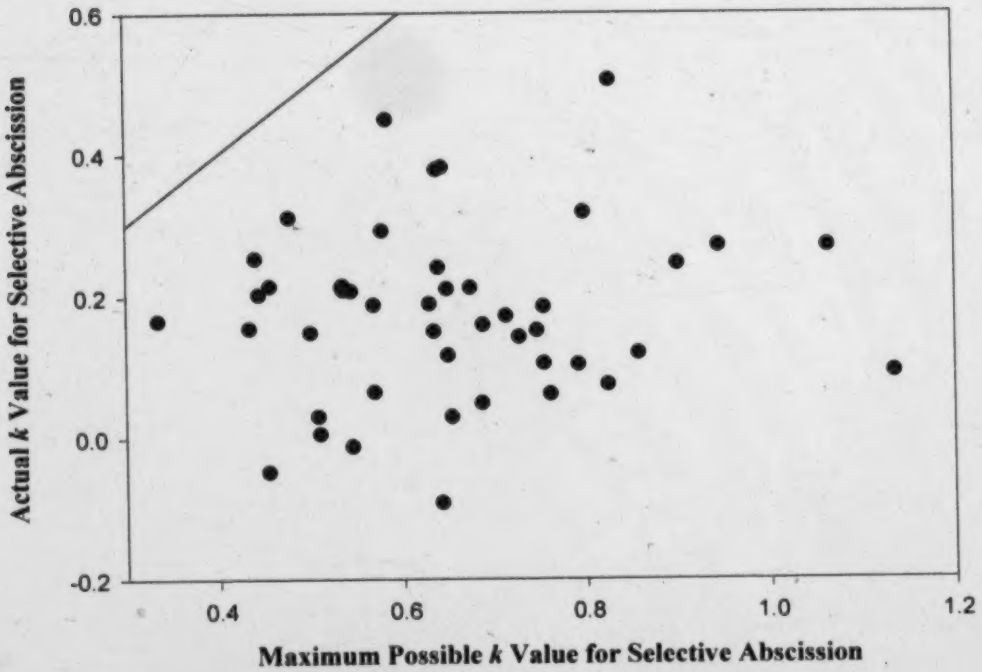

Figure 2.10. Each inflorescence's *k* value for selective abscission is plotted against its maximum possible *k* value (that which would have resulted from exact retention of the flowers with the fewest eggs) n=47 inflorescences. The line represents a 1:1 relationship. Actual *k* values are not correlated with maximum possible *k* values (linear regression not shown, maximum possible *k* value is insignificant predictor of actual *k* value: $p=0.16$, $df=44$, $r^2=.023$).

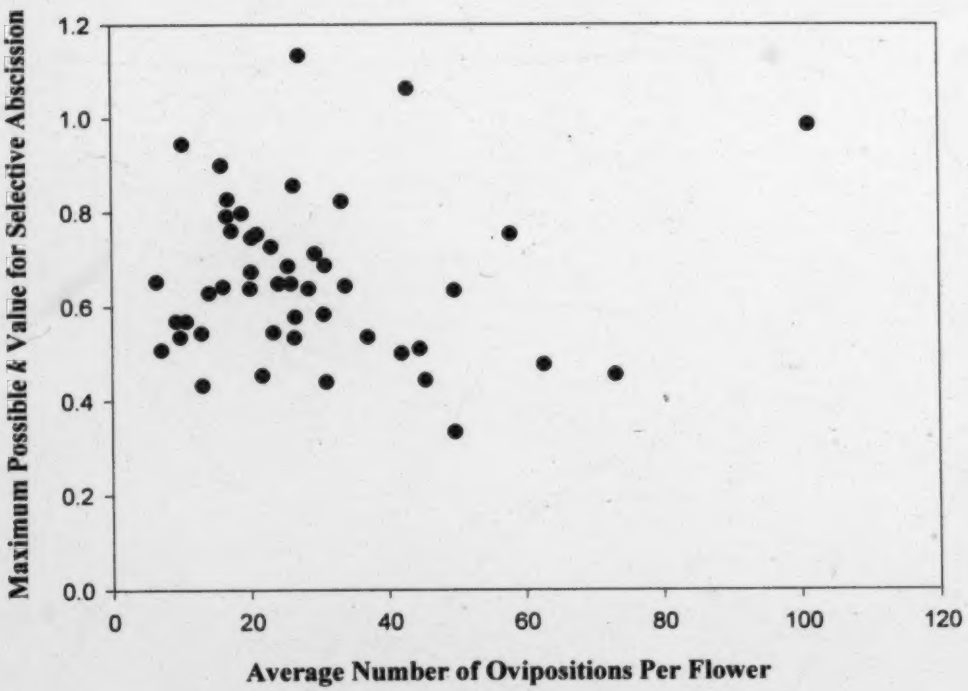

Figure 2.11. An inflorescence's maximum possible *k* value for selective abscission (that which would have resulted from retention of exactly the flowers with the fewest ovipositions) does not increase with the mean number of eggs per flower (linear regression insignificant: p=0.09, d.f=44, r^2=.044). n=47 inflorescences.

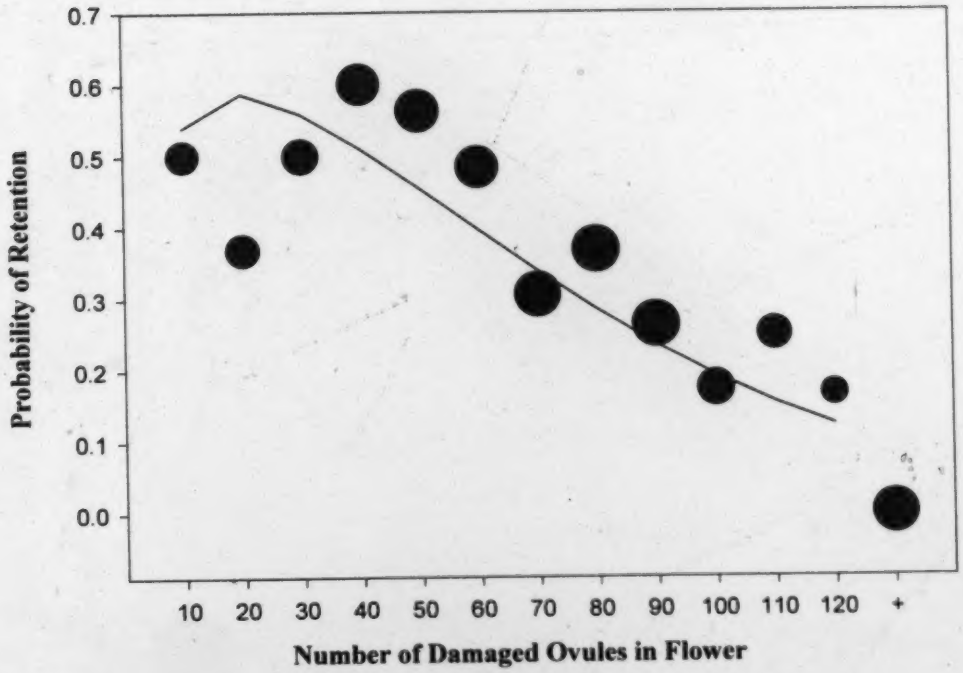

Figure 2.12. Retention (proportion of visited flowers retained as fruit) as a function of number of damaged ovules per flower (Experiment 2). Symbol size is proportional to sample size. Line represents logistic regression ($y=1/(1+e^{-(1.354-0.027x-9.260/x)})$ for 354 visited flowers and fruit, $\chi^2=42.4$, d.f.=2, $p<0.001$.

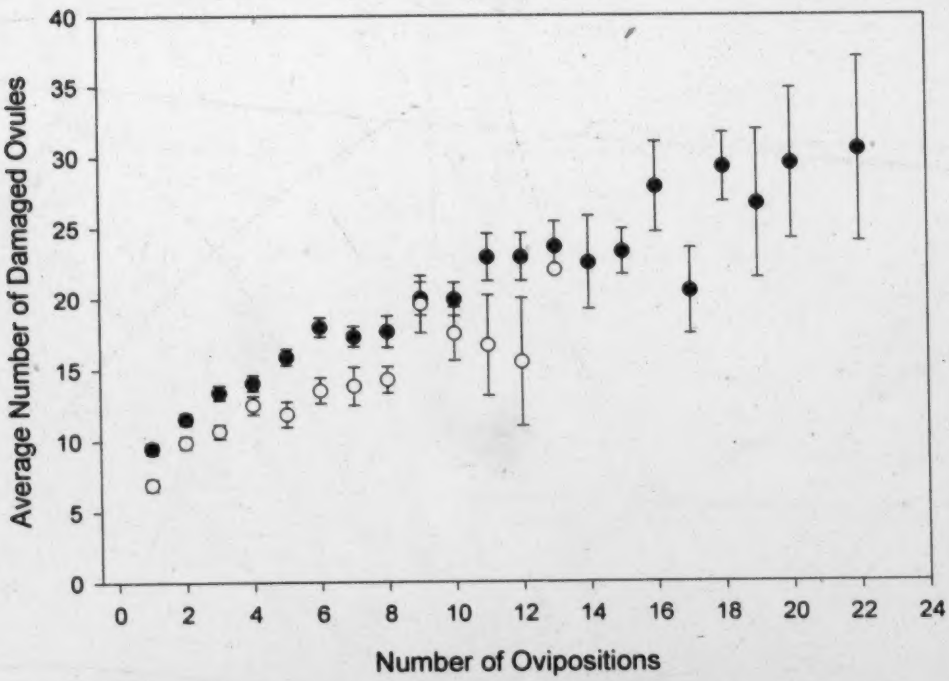

Figure 2.13. Number of damaged ovules in a locule as a function of number of ovipositions. Black circles represent abscised flowers (n=1194). Empty circles represent retained fruit (n=614). Lines represent standard error. The relationship between damage zone and oviposition number in locules is significantly different between abscised and retained flowers (n= 1808, df=1, F=68.6, $p<0.001$). Second order terms are significant in regressions for both abscised and retained flowers ($p<0.001$ for both):. Abscised flowers: $y= 8.429 + 1.583x - 0.033x^2$, n=1194, adj. $r^2=0.30$, F=253.6, $p<0.001$. Retained flowers: $y= 5.842 + 1.793x - 0.071x^2$, n=614, adj. $r^2=0.26$, F=60.5, $p<0.001$

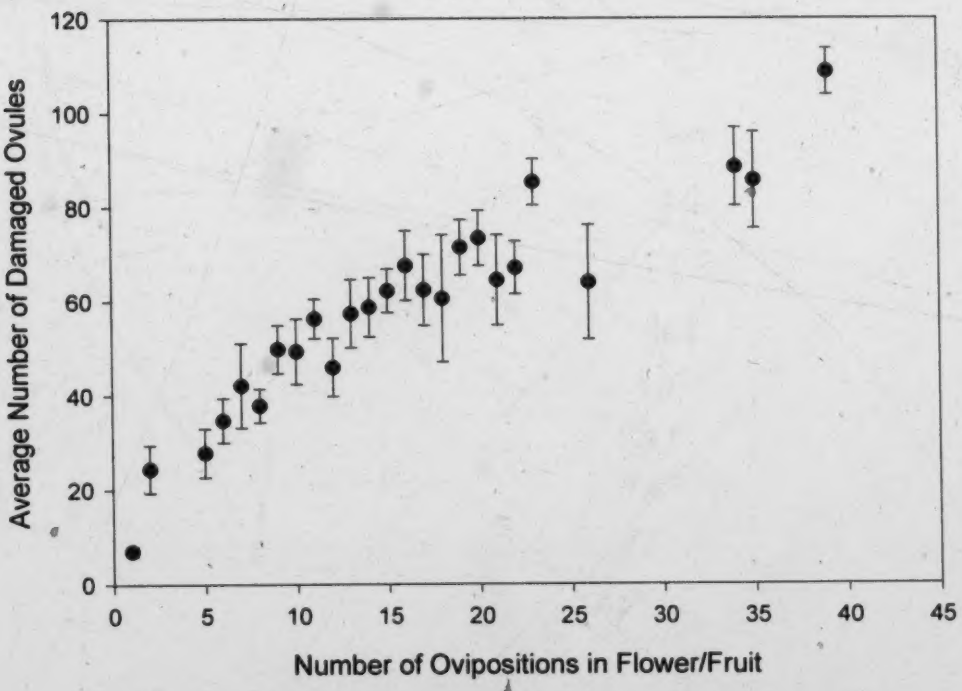

Figure 2.14. Number of damaged ovules in a flower as a function of number of ovipositions. This graph differs from 2.13 by looking at flowers rather than locules, and by combining abscised flowers and retained fruit. In other words, this represents all of the flowers that the plants had, from which they selected those to be retained. Each point represents 6 to 21 flowers, and the lines represent standard error. Regression on raw data: $y=20.41 + 2.69x - 0.01x^2$, d.f. = 354, F=297.0, $p<0.001$, adj $r^2= 0.63$

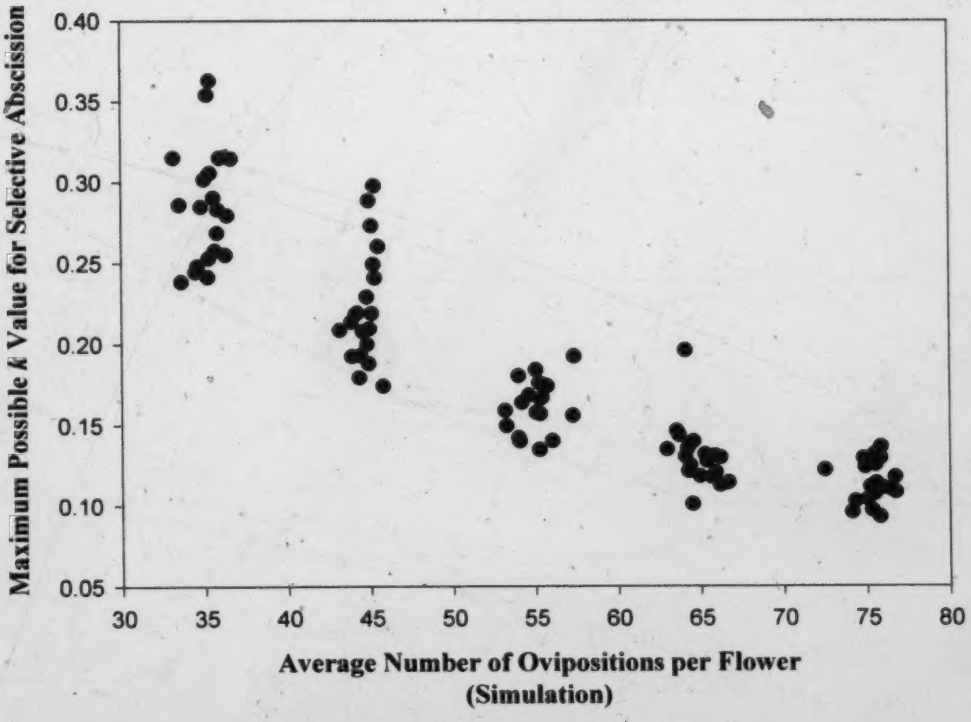

Figure 2.15. Inverse density-dependence of *k* values in hypothetical inflorescences with normal frequency distributions of oviposition numbers. Each point represents one inflorescence with a mean number of ovipositions per flower (ca. 35 – 75) and a constant standard deviation of about 10.

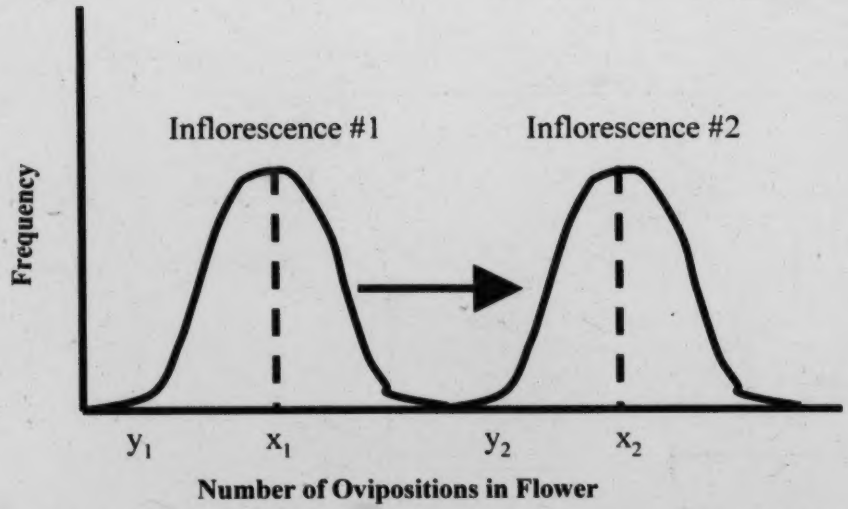

Figure 2.16. Schematic to illustrate the principle behind the inverse density-dependence shown in Fig.2.15. Let each normal curve represent the frequency distribution of ovipositions per flower in one inflorescence. Inflorescence # 2 has a larger mean number of ovipositions per flower than inflorescence #1 ($x_2 > x_1$). Let y_1 and y_2 represent the mean of the lowest 10% of values for inflorescence #1 and #2, respectively. $x_2 - y_2 = x_1 - y_1$ but $(x_2 - y_2)/x_2 < (x_1 - y_1)/x_1$. The difference between the smallest oviposition levels and the mean oviposition levels has declined proportionately in the second inflorescence, and this is why *k* selective abscission declines with mean oviposition.

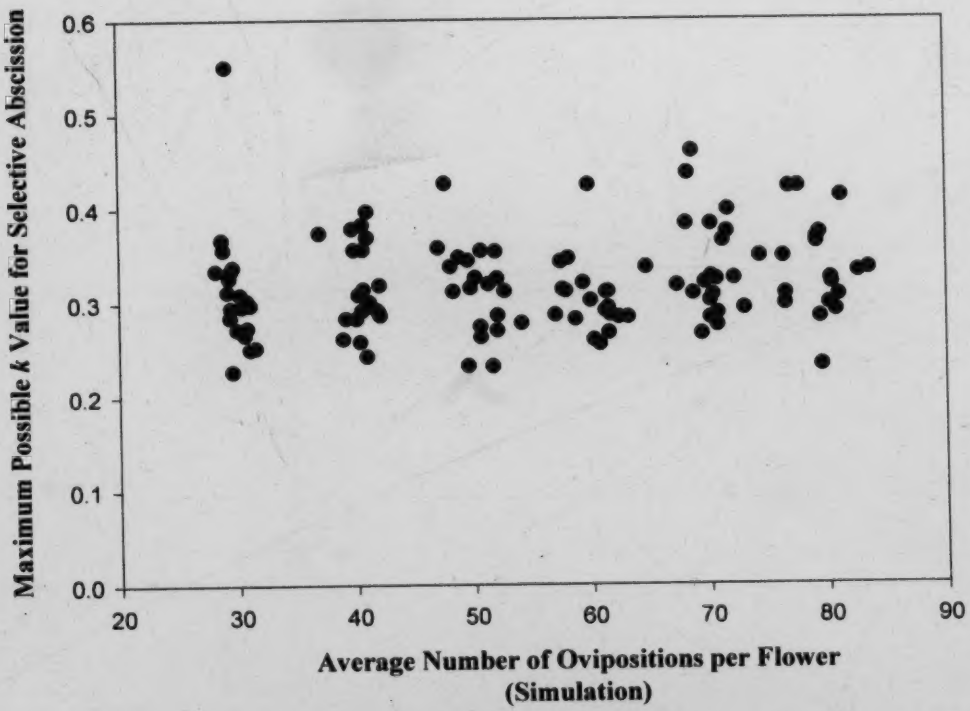

Figure 2.17. Density-independence of moth egg mortality (as measured by *k* values for selective abscission) on hypothetical inflorescences. Each point represents one hypothetical inflorescence with a normal frequency distribution of number of eggs per flower. The standard deviation of number of eggs per flower on each inflorescence is 0.30 x the mean number of eggs per flower. When standard deviation of egg numbers on a plant increases linearly with mean egg number on a plant, selective abscission is density- independent.

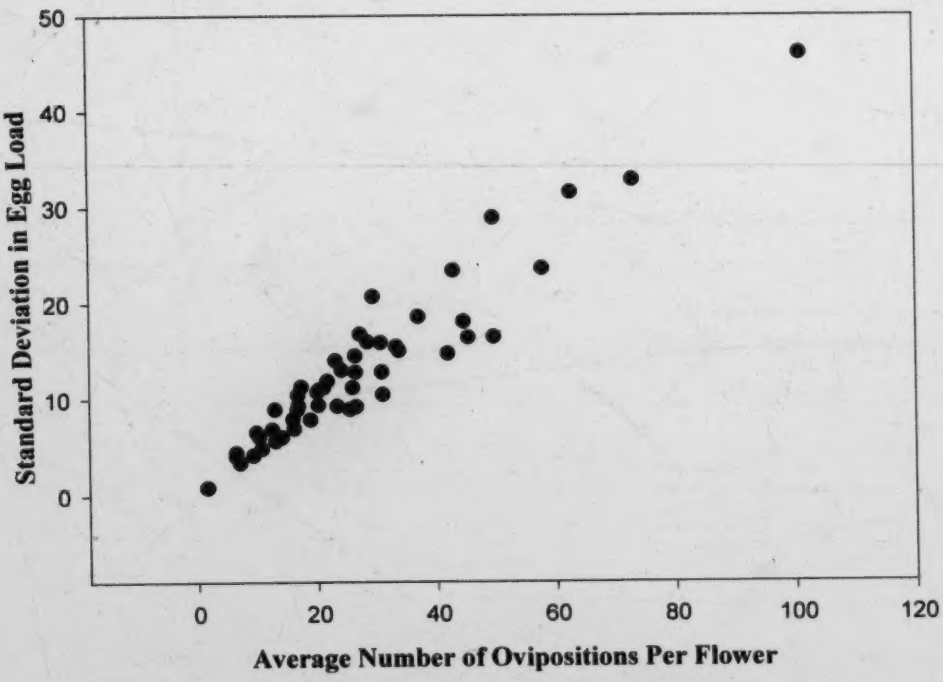

Figure 2.18. The standard deviation of number of ovipositions in the flowers of an inflorescence increases linearly with the average number of ovipositions per flower in an inflorescence. Each point represents one inflorescence, n=52.

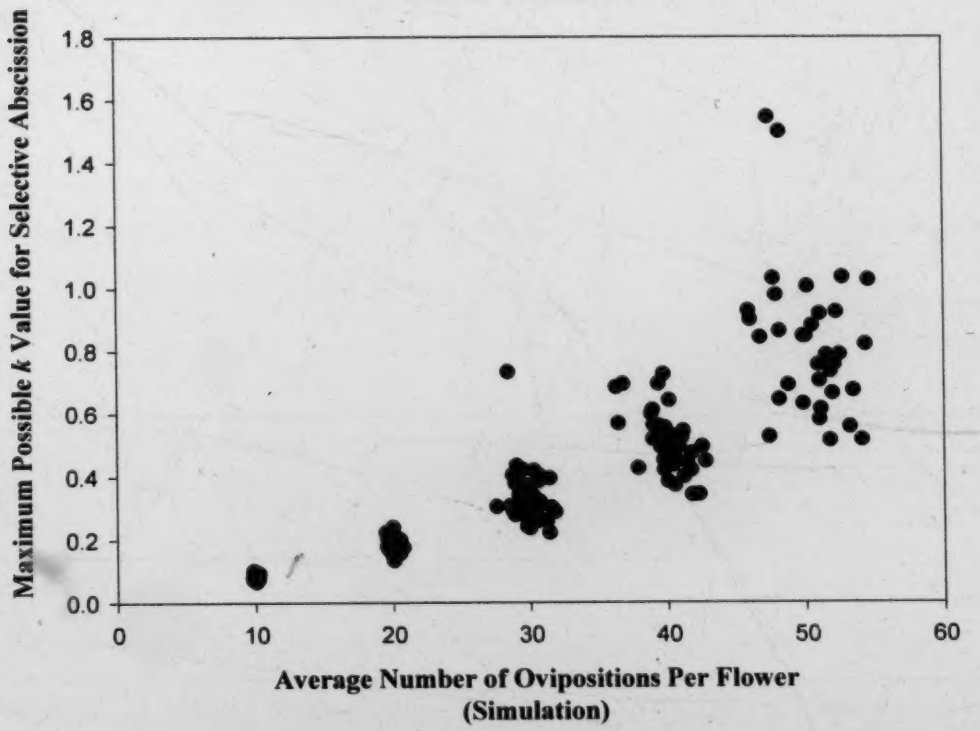

Figure 2.19. Density-dependence of selective abscission moth egg mortality on 195 hypothetical inflorescences. Each inflorescence has a randomly generated normal frequency distribution of eggs per flower with standard deviation = $0.01x^2$, where x = mean number of eggs per flower.

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Appendix 2.1

Explanation of results found with simulated inflorescences

Let there be two inflorescences with one hundred flowers each. Suppose the frequency distribution of eggs per flower on an inflorescence is normal. Let x_1 be the mean number of eggs per flower in yucca 1, and x_2 be the mean number of eggs per flower in yucca 2 (as Fig. 2.16). Let y_1 be the mean number of eggs in yucca 1's 10 flowers with the fewest eggs (i.e., the flowers the plant would ideally retain). Let y_2 be the mean number of eggs in yucca 2's 10 flowers with the fewest eggs. Let d (for difference) be x_2-x_1. Let f(d) be y_2-y_1. (Thus, if variance is constant as mean increases, f(d) equals d.)

Then, k_1 selective abscission = $\log (x_1/y_1)$
k_2 selective abscission = $\log (x_2/y_2) = \log ((x_1+d) / (y_1+f(d)))$

To answer the question does *k* selective abscission increase with mean, we ask is $k_2 > k_1$?

$$\begin{aligned} k_2 - k_1 &= \log ((x_1+d) / (y_1 + f(d)) - \log (x_1/y_1) \\ &= \log ((x_1+d) / (y_1 + f(d)) + \log (y_1/x_1) \\ &= \log (y_1/x_1) + \log ((x_1+d) / (y_1 + f(d)) \\ &= \log (y_1/x_1) + \log ((x_1/f(d) + d/f(d)) / (y_1/f(d) + 1)) \end{aligned}$$

The first term of this equation is a negative constant, since y1 < x1. As d increases to infinity (i.e., as mean increases), the second term goes to log (d / f(d)).

1. If variance is unchanging with mean, f(d) = d. Thus, $k_2 - k_1$ goes to a negative constant plus log (d/d), which equals a negative constant plus 0. This means $k_2 < k_1$. If the number of eggs per flower is normally distributed on an inflorescence and the standard deviation of eggs per flower is constant regardless of mean, then *k* selective abscission will decrease with mean number of eggs per flower on an inflorescence (as in Figure 2.15)

2. What about if the standard deviation in number of eggs per flower increases with the mean number of eggs per flower on an inflorescence? If $s = c_1 * m$ (i.e., standard deviation increases linearly with mean at rate constant, c_1), simulations suggest that $f(x) = c_2 d$. In other words, if s increases linearly with m, f(x) also increases linearly (although not with the same constant) with d. I do not see an obvious relationship between c_1 and c_2.

Therefore, $k_2 - k_1$ goes to a negative constant plus log $(d / c_2 * d)$, which equals a negative constant plus log $(1/c_2)$. In other words, the difference between k_2 and k_1 is independent of d, which means k selective abscission neither increases or decreases with the mean number of eggs per flower on an inflorescence when standard deviation increases linearly with the mean (as in Figure 2.17).

Chapter 3

The Role of Egg Mortality in the Regulation of the Yucca – Yucca Moth Mutualism

3.0 Introduction

Many mutualisms are best described as balanced reciprocal exploitation in which each partner would increase its fitness by increasing its exploitation of the other (Janzen 1983, Bronstein 1994, Herre *et al.* 1999). Learning what sorts of mechanisms limit exploitation in these relationships is central to improving our understanding of mutualism (Axelrod and Hamilton 1981, Bull and Rice 1991, Bao and Addicott 1998, Doebli and Knowlton 1998, Herre *et al.* 1999, Yu 2001). The objective of this study is to identify mechanisms that promote the stability that is observed in the obligate pollination – seed predation mutualism between *Tegeticula altiplanella* (yucca moth) and *Yucca kanabensis*.

Except in rare instances (Dodd and Linhart 1994), yucca moths are the sole pollinators of yuccas (Riley 1892, James *et al.* 1993). The moths lay their eggs in yucca flowers and the larvae that hatch from these eggs consume an average of only 15% of developing seeds in a fruit (Addicott 1986). Complete consumption of the seeds of a yucca fruit by moth larvae is very rare (Humphries 1998). In the absence of any trade-offs or constraints, natural selection should favour more complete utilization of the seed resource by moths. What constraints or trade-offs limit seed consumption to such a low level?

Seed consumption could be regulated by density-dependent mortality of moths. If mortality took place during the egg stage, in flowers, it would directly limit the number of larvae feeding in a fruit. Exactly compensating density-dependent egg mortality would prevent the number of larvae feeding in fruit from exceeding a certain number, regardless of the number of eggs laid. Mortality at a life history stage other than the egg stage could limit larval feeding by reducing the number of adult moths visiting flowers and, hence, the number of eggs laid per flower. Regulation of the adult moth population could potentially maintain it at a density at which flowers have reasonably low numbers of eggs laid per flower. In support of the moth population regulation hypothesis, the *T. altiplanella* population near Kanab appears to be relatively stable from year to year (Addicott 1998). This stability is indicated by a negative correlation between the proportion of rosettes flowering and the proportion of flowers that are visited by moths

(Addicott 1998). In the previous chapter I rejected the hypothesis that *T. altiplanella* population size is regulated by selective abscission. Moth eggs in abscised flowers always perish and this is a significant source of mortality, but egg mortality resulting from selective abscission was not density-dependent, and therefore not population regulating.

In this chapter I investigate egg mortality in developing fruit. Addicott and Bao (1999) have observed highly density-dependent mortality of *T. altiplanella* eggs in developing *Y. kanabensis* fruit. The mortality takes place before the larvae reach their 2nd instar, which means it occurs before they begin feeding on developing seeds. Therefore, although competition for seeds and cannibalism can be ruled out, the cause of this egg mortality is not known.

Female *T. altiplanella* moths damage some ovules in the process of oviposition (Addicott 1986, Addicott and Bao 1999). Often, multiple moths visit the same flower and they all oviposit in the same region of the flower. This creates a "damage zone" of contiguous ovules that do not develop into seeds (Figure 2.5). Some of the moth eggs wind up being inside the damage zone and I hypothesized, based on initial observations, that they are less likely to survive than are eggs outside the damage zone. The specific questions I address with this experiment are:

1. How much *T. altiplanella* egg mortality takes place in retained *Y. kanabensis* fruit?
2. At what stage does mortality take place? (This might provide insight into the cause of mortality.)
3. Do eggs in the damage zone of a locule experience higher mortality than eggs outside the damage zone? (i.e., Is the damage zone associated with a source of egg mortality?)
4. How much of the egg mortality that takes place in fruit can be attributed to mortality of eggs inside the damage zone? (i.e., Is the damage zone *the primary* source of egg mortality in fruit?)
5. Is egg mortality in retained fruit density-dependent and, therefore, potentially a population regulating factor?

I attempted to answer these five questions with a field experiment in which I harvested yucca flowers at different stages. Since yuccas selectively abscise flowers with many damaged ovules (see section 2.2.2.), four day old flowers that have been randomly harvested can be expected to have more damaged ovules per oviposition than fruit that the plant has selectively retained (see section 3.1.2.1 for elaboration). This bias would confound a test of whether the damage zone is a source of egg mortality. To eliminate this selective abscission bias I removed all but 10% of flowers on experimental yuccas and then randomly assigned harvesting ages to the remaining flowers. In this way I "forced" the yuccas to retain the fruit that I randomly selected, instead of allowing them to selectively retain fruit with small damage zones.

After addressing my research questions with this experiment, I compare the egg mortality rate and the proportion of larvae in the damage zones of this experiment's "forced" fruit to those in the "natural" fruit collected in another experiment ("Selective abscission", 2.1.2). In the "Selective abscission" experiment of the previous chapter, yuccas had their full inflorescence and exercised selective abscission. I compare the survival of larvae in the fruit of the two experiments to confirm the predicted selective abscission bias and to check for treatment effects (unintended effects of 90% flower removal) in the "forced" fruit.

Finally, I consider the following questions. How does egg mortality in fruit affect seed production by yuccas? Is the observed rate and density-dependence of egg mortality sufficient to explain the low rate of seed predation reported by others (Addicott 1986, Humphries 1998)? If so, what role does the regulation of moth population size play in regulating the mutualism costs experienced by *Y. kanabensis*?

3.1 Methods

3.1.1 Study Sites and Organisms

I studied the interaction between *Yucca kanabensis* McKelvey (Agavaceae) and its obligate pollinator / seed predator moth *Tegeticula altiplanella* Pellmyr (Prodoxidae). The taxonomic status of yucca populations near Kanab is unresolved (McKelvey 1947, Webber 1953, Cronquist *et al.* 1977) and, following Addicott (1998), I use McKelvey's (1947) classification, using the name *Y. kanabensis* for yuccas between Kanab and Zion National Park.

My field experiments took place over two field seasons at two sites near Kanab, Kane County, Utah. The first field season was June 13th to July 15th, 1999. The second was May 23rd to July 7th, 2000. The "Yellowjacket" site is located on the road to Coral Pink Sand Dunes State Park near Yellowjacket Ranch (37° 7' 30" N, 112° 40' 45" W, 1800m elevation). The "Red Knoll" site is at the base of Red Knoll, on its eastern side, about 15 kilometres northwest of Kanab (37° 9' 12" N, 112° 38' 0" W). The vegetative community at both sites is dominated by *Y. kanabensis*, *Artemesia tridentata* Nuttall, *Quercus gambelli* Nuttall, *Juniperus monosperma* Engelmann, and *Wyethia scabra* Nuttall.

Yucca kanabensis is a long-lived polycarp that grows on open sandy soil. It produces a raceme 1 – 2 metres tall with approximately 50 – 150 flowers which bloom in 1 – 3 overlapping waves from the base to the apex of the inflorescence (Addicott 1998). Each flower is hermaphroditic and is attractive to moths and receptive to pollen for two nights. Fruit production in *Y. kanabensis* is usually resource-limited, meaning that it is limited by resources other than pollen (versus "pollen-limited"). Typically, yuccas mature 6-10% of their flowers into fruit (Addicott 1985, 1998). As well as reproducing from seed, *Y. kanabensis* reproduces vegetatively, with clones having up to 44 rosettes (Addicott 1998).

After mating in the spring, female yucca moths collect pollen, oviposit into fresh flowers, and then actively pollinate the flowers with their unique maxillary tentacles. Yucca moths depend entirely on yuccas for reproduction because they only oviposit in yucca flowers and the developing seeds of yucca fruit are the sole food source for their

larvae. Eggs hatch after 9 – 11 days (James 1998, personal observation) and larvae feed inside fruit for about thirty days (Humphries 1998) before chewing out of the fruit, dropping to the ground and entering pre-pupal diapause in the soil, where they overwinter.

Yucca kanabensis has two pollinators: *T. altiplanella*, which occurs at my sites, and *T. superficiella*, which does not occur at my sites. *Tegeticula altiplanella*, also referred to as "deeps", oviposit through the carpel wall into the locular cavity, hence their common name. In contrast, *T. superficiella* ("shallows") oviposit into the carpel wall, damaging no ovules. Oviposition by *T. altiplanella* damages some ovules, which never develop into seeds. *Tegeticula altiplanella*'s oviposition behaviour is highly stereotyped; all oviposit about 1/3 of the way down the long axis of the ovary from the apical end, creating a contiguous row of damaged ovules which I call the "damage zone" (Figure 2.5). It is this aspect of *T. altiplanella* oviposition behaviour that may lead to the demise of eggs in fruit. The immediate environment around an egg in the damage zone is different from the environment around an egg outside the damage zone physically and probably chemically. Egg mortality in the damage zone might be expected to be density-dependent; as more moths oviposit in the same locule, the ovules around any given egg are more likely to become damaged. One can also imagine that ovipositors might pierce previously laid eggs during oviposition, but eggs are frequently seen side by side, so it does not seem likely that this occurs with any regularity. I have also observed that eggs can be prodded a fair bit with sharp tweezers (albeit a very different shape and size from an ovipositor) and do not rupture, indicating that they are not extremely fragile.

3.1.2 Field Methods

Mortality of *T. altiplanella* eggs in *Y. kanabensis* fruit can not be studied directly, because observation of eggs requires cutting into a fruit's carpels, which destroys the sample and kills the eggs. This means one cannot track the survival of individual eggs and larvae through time. However, when a moth oviposits into a locule, her ovipositor leaves a track. Oviposition tracks can be counted to estimate the number of eggs laid in a locule. Addicott (unpubl.) has found a 1:1 correlation between oviposition holes and *T. altiplanella* eggs in fresh *Y. kanabensis* flowers. Oviposition holes begin to stretch and

overlap in older fruit, rendering counts less accurate in fruit older than 14 days (personal observation).

A flower or fruit can be dissected at any age (up to about 14 days) to count ovipositions (the number of eggs laid) and the number of surviving eggs or larvae. The survival rate of eggs or larvae can thereby be determined in flowers and fruit. Additionally, the locations of eggs in flowers can be compared to the locations of larvae in fruit: Do eggs occur inside the damage zone of a locule more frequently than larvae do? This would indicate that eggs laid in the damage zone experience higher mortality than eggs outside the damage zone.

3.1.2.1 Selective Abscission Bias

Yucca kanabensis selectively abscises flowers with many damaged ovules and many eggs, but it is the damaged ovules that appear to be the cue used by the plant for abscission (see section 2.2.2.). The relationship between the number of eggs and the number of damaged ovules in a locule varies, with some flowers having many damaged ovules per oviposition and some having relatively small damage zones for the same number of eggs. One expects fruit that have been selectively retained based on damage zone size to have fewer damaged ovules per egg than the average flower (Figure 3.1).

To determine if eggs were more likely to perish inside the damage zone, I wanted to compare the proportion of eggs inside the damage zone of four day old flowers to the proportion of larvae in the damage zone of 14 day old fruit:

Locules of four day old flowers:

number of eggs in damage zone / total number eggs in locule

Locules of 14 day old fruit:

number of larvae in damage zone / total number larvae in locule

If eggs are equally likely to die inside the damage zone as outside the damage zone, then the total number of larvae will be smaller than the total number of eggs, and the number of larvae in damage zone will be smaller than the number of eggs in the damage zone, but the proportion will be, on average, the same. If, on the other hand, larvae occur in the damage zone with a much lower frequency than eggs do, this would

indicate that eggs in the damage zone experience more mortality than eggs outside of the damage zone.

However, because selectively retained fruit have smaller damage zones than the average flower, a comparison of selectively retained fruit to randomly harvested flowers would yield the same result: a lower proportion of larvae in the damage zone of fruit than the proportion of eggs in the damage zone of flowers. Therefore, selective abscission would confound the test of the damage zone being a source of egg mortality. In order to minimize yuccas' selective abscission opportunity I removed 90% of flower buds and randomly assigned harvesting ages to the remaining flowers. As it turned out, fewer than 10% of flowers were retained, on average at my sites and so there was still a small amount of selective abscission bias (Figure 3.2), but not sufficient to be problematic, because it is not sufficient to explain the very strong pattern seen in Figure 3.5.

3.1.2.2 Incomplete Ovule Fertilization

Incomplete ovule fertilization is another variable that might affect egg and larval survival. If a retained fruit has many unfertilized ovules, eggs beside the undeveloped ovules may perish. Also, even if 90% of buds were removed, if any of the remaining flowers did not receive adequate pollen, the flower would be abscised, thereby reducing my sample size. To prevent these problems, I hand-pollinated each flower the morning after it opened, which ensured that each flower had sufficient cross pollen for full ovule fertilization (see 2.1.3.2 for hand pollination protocol).

3.1.2.3 Field Method Details

In 1999 I selected six inflorescences at the Yellowjacket site that were all at the bud stage (one was eaten by cattle, five survived). In 2000 I selected 24 inflorescences at Red Knoll (nine were eaten by cattle, 15 survived) and 10 inflorescences at Yellowjacket that were at the bud stage (all survived).

I removed all but 10% of the buds on inflorescences, leaving buds that were all on the same flower wave (the first or second wave) and had similar axil positions as one another. Other investigators have removed flowers and have not found this to negatively affect fruit set (Wilson and Addicott 1998, Humphries 1998). I labelled buds with a

Sharpie® Fine Point Permanent Marker and used a stratified random design to assign harvesting treatments to them. Flower buds were stratified according to their axil position, meaning that if there were four treatments (treatment=harvesting age) then the lowest four buds would include one bud for each treatment, the next four buds up the inflorescence would include one bud for each treatment, and so on. The purpose of this stratification was to minimize the potential confounding effects of axil position.

In 1999, the four treatments were: harvest four days after anthesis, six days after anthesis, eight days after anthesis, and 10 days after anthesis. I thought that 10 day old fruit would contain first instar larvae, but they had about half eggs and half first instar larvae. For my second season (May-July 2000), I changed the harvesting ages to four days after anthesis, eight days after anthesis, and 14 days after anthesis.

Four days after anthesis is the soonest that damaged ovules can be clearly discerned from undamaged ovules. I assumed that there would be minimal egg mortality in four day old flowers; the purpose of collecting four day old flowers was to see what proportion of eggs were in the damage zone. Fourteen day old fruit contained 1st and some 2nd instar larvae that had just begun chewing on the seed they were on, but had not yet moved from where they hatched.

I hand-pollinated each flower the morning after it opened. Once all of the flowers on an inflorescence had been open for two nights, I enclosed the inflorescence in a mesh cage made of window screening to discourage browsing by cattle. I checked inflorescences daily and harvested flowers when they reached their assigned harvesting age: four, eight, or 14 days. I did not use flowers that abscised.

3.1.3 Dissection Methods

Each flower has six distinct locules (chambers of ovules) that are separated by a locule wall (septum). Eggs are laid inside locules, each locule with eggs has a damage zone, and mortality takes place before larvae begin moving (and thus before they might cross locules). Mortality in one locule is therefore independent of mortality in adjacent locules. Therefore, the relevant sample unit is the locule.

I estimated the number of eggs laid in locules by counting ovipositions. To count ovipositions, thin sections of the carpel wall were sliced away with a scalpel, revealing

the oviposition tracks. I dabbed blue food dye diluted in water on to the freshly sliced carpel, which assisted my counting by pooling up in the tracks and making them more visible.

After counting the ovipositions in a flower or fruit, I dissected it to count the eggs or larvae and the damaged ovules. I also recorded how many of the eggs or larvae were inside the damage zone (i.e. beside a damaged ovule). Dissections were done using a binocular light microscope, at about 10x magnification.

Analyses are based on locules that had at least one oviposition: In 1999 there were 27 four day old locules, 30 six day old locules, 65 eight day old locules, and 49 ten day old locules, and in 2000 there were 342 four day old locules, 322 eight day old locules, and 325 fourteen day old locules.

3.1.4 "Selective Abscission Fruit" for Comparison

In 2000, fruit from the previously described "selective abscission" experiment (section 2.1.2) were also dissected to count ovipositions, larvae, damaged ovules, and to record the locations of larvae. These fruit are from inflorescences that had their full suite of flowers and that exercised selective abscission (n=1066 locules). They were not hand-pollinated, but their ovules were all fertilized by natural pollination.

3.2 Results

3.2.1 How much egg mortality occurs in retained fruit?

On average, only 56.5% of eggs survived to 14 days, with an average of only two larvae surviving in a locule. Mortality was density-dependent, as is indicated by the significant second order term in the relationship between number of eggs laid and number of surviving larvae ($p<0.001$, Figure 3.3). The proportion of eggs surviving to 14 days decreased from approximately 0.60 when one egg was laid in a locule to approximately 0.39 when 14 eggs were laid.

3.2.2 At what stage does mortality take place?

In this experiment I dissected four, eight, and 14 day old flowers. The purpose of dissecting four day old flowers was to determine the proportion of eggs that are laid inside the damage zone, for comparison with the proportion of larvae that survive in the damage zone of fruit (section 3.2.3). Four days after anthesis is the soonest the damaged ovules are distinguishable from the ovules growing around them. I assumed that there would be no egg mortality during the first four days in a flower and that, even if there was a small amount of egg mortality, those recently demised eggs would still be visible and counted. However, in four day old flowers there were only 71.0% as many eggs as ovipositions, on average, with this rate being slightly density-dependent ($p<0.001$ for 2nd order term, Figure 3.4).

This result suggests that some egg mortality takes place during the first four days that eggs are in a flower. I previously hypothesized that during oviposition, a moth might damage previously laid eggs in a locule. Since undamaged eggs are frequently seen side by side or near one another, I expect that this is a minor source of mortality. It is also possible that I was not able to find all of the eggs, which are very small, translucent and can be ruptured during dissection. Early egg mortality and unfound eggs can not be disentangled, so I will not use these data to estimate the rate of mortality occurring by age four. Based on the reasonable assumption that eggs are equally easily found in the damage zone as outside the damage zone, I use the data from four day old flowers only to estimate the proportion of eggs in the damage zone (section 3.3.3).

Eight day old flowers had the same number of eggs in them for a given number of ovipositions as four day old flowers (ANCOVA: F=2.146, n=789, df=1, p=0.14). However, this does not necessarily mean that no egg mortality takes place between age four and eight days. My observation is that some eggs become withered and as they die; some eggs, however, that were clearly dead (because they were in the damage zone of 14 day old fruit, alongside larvae just outside the damage zone) were perfectly turgid. Nathan Forsyth has also found *T. baccatella* live and dead eggs indistinguishable in *Y. baccata* fruit (pers. com.). Since live eggs are apparently indistinguishable from dead eggs, I can not estimate mortality rates during the egg stage.

To summarize, the amount of *T. altiplanella* mortality that takes place by the time larvae are 14 days old is shown in Figure 3.3. The question of precisely when this mortality occurs remains unanswered, because eggs are harder to find than larvae, and because at least some dead eggs are indistinguishable from live eggs. However, it is important that the mortality takes place before larvae begin feeding on seeds, for this eliminates the possibility that the mortality is due to inadvertent cannibalism. It also means that this mortality probably limits the total amount of seed predation in fruit. Although there may some density-dependence in yucca moth larval feeding rates (James 1998), the rare fruit that do contain many surviving larvae do have all of their seeds consumed (Rau 1945, Addicott 1986).

3.2.3 Do eggs in the damage zone of a locule experience higher mortality than eggs outside the damage zone?

On average, 28.6% of eggs in locules of four day old flowers are in the damage zone, and this is density-dependent (Figure 3.5). On average, only 1.1% of larvae in locules of 14 day old fruit are in the damage zone, and this is not density-dependent (Figure 3.5). The proportion of eggs (four days old) in the damage zone is significantly higher than the proportion of larvae (14 days old) in the damage zone, controlling for number of ovipositions (ANCOVA, $p<0.001$).

Eggs laid in the damage zone almost never survive to 14 days, and the proportion of larvae in the damage zone is consistently close to zero regardless of the number of eggs laid.

3.2.4 How much of the egg mortality that takes place in fruit can be attributed to mortality of eggs inside the damage zone?

Complete mortality of eggs in the damage zone appears to be sufficient to explain the mortality rates observed in 14 day old fruit. If one predicts that all of the eggs observed in the damage zones of four day old flowers will perish, and assumes that all of the eggs observed outside the damage zones of four day old flowers will survive, the predicted number of surviving larvae matches the observed number of larvae surviving in 14 day old fruit (Figure 3.6). An ANCOVA supports the hypothesis that there is no difference between the predicted number of surviving larvae based on the damage zone hypothesis and the actual number of surviving larvae in 14 day old fruit ($p=0.75$).

3.2.5 Is Egg Mortality Density-dependent and a Potential Regulating Factor?

The statistically significant and negative second term in the regression of number of surviving larvae on number of eggs laid indicates that larval mortality in developing fruit is density-dependent (Figure 3.3). But how strong is this density-dependence? Does it place an effective limit on the number of larvae feeding in fruit? As the number of eggs laid in a locule increased up to seven eggs, the number of larvae surviving also increased (Figure 3.3). When more than seven eggs are laid in a locule the pattern is less clear, as there is more variation around the regression line. However, the regression line suggests that as the number of eggs in a locule increases, the number of larvae that survive generally does not exceed three. The regression suggests that egg mortality in retained fruit may be undercompensating at low egg densities, and then become perfectly compensating, keeping the number of larvae surviving (and feeding) in locules to an average of three.

3.3 Discussion

The purpose of this study was to determine how much *T. altiplanella* egg mortality takes place in retained *Y. kanabensis* fruit, whether this mortality is density-dependent, and to test the "damage zone hypothesis" that eggs laid beside oviposition-damaged ovules have higher mortality than eggs laid beside intact ovules.

I confirmed that there was density-dependent egg mortality in fruit (Figure 3.3), but egg mortality rates were lower in my experimental fruit than the rates reported by Addicott and Bao (1999). Eggs laid in the damage zone of flowers rarely survived (Figure 3.5), and this mortality was sufficient to explain the observed difference between the number of four day old eggs in flowers and the number of fourteen day old larvae in fruit (Figure 3.6). In other words, the mortality that took place between day four and day 14 in fruit could be entirely accounted for by 100% mortality of eggs in the damage zone. The source of egg mortality that occurred before flowers were four days old could not be analyzed because the damage zones were not visually distinguishable from undamaged ovules until flowers were four days old.

Removing 90% of buds did sufficiently reduce the selective abscission bias (Figure 3.2), allowing for useful comparison between flowers and fruit (Figures 3.5, 3.6). However, the experimental design also had unpredicted side effects that render the estimated egg mortality rate based on experimental fruit flawed. This side effect is shown in Figures 3.7 and 3.8. Figure 3.7 shows that the experimental fruit ("forced fruit", because they were forced to be retained) had significantly higher larval survival than did natural fruit collected during the "Selective Abscission" experiment of Chapter 2. Figure 3.8 shows the apparent reason for the higher larval survival; forced fruit had significantly fewer damaged ovules per oviposition than did natural fruit. Accordingly, the fruit collected by Addicott and Bao (1999) were also from inflorescences that did not have flowers removed and that went through the natural selective abscission process. As mentioned, Addicott and Bao's (1999) fruit had lower larval survival than did my "experimental fruit". The larval survival rates they observed were closer to those I observed in my "natural fruit".

In hindsight this side effect is perhaps not surprising, because it is well known that plants do shift their resource allocation when flowers are removed (Stephenson 1981). Numerous writers report that artificially thinning flowers and young fruit from trees results in the allocation of some of the surplus resources to the remaining fruit, resulting in more fruit as well as increased fruit and seed size (cited in Stephenson 1981). In other words, experimental yuccas that had 90% of buds removed did not invest resources into those flowers that they naturally would have, and instead they probably allocated those resources into the remaining flowers. Additional resources allocated to the remaining flowers probably resulted in the flower developing ovules that were slightly damaged by oviposition that would otherwise have been aborted early on, reducing the size of the damage zone. Inflorescences with 90% of buds removed did have significantly higher retention than inflorescences with no flowers removed (8% retained vs. 6% retained, $p=.011$, $df=1$, $n=60$), which supports the hypothesis that removing buds resulted in more resources allocated to remaining flowers.

Even if shifted resource allocation is not the entire reason for the observed experimental bias, it is clear that experimental "forced" fruit had smaller damage zones than natural fruit (Figure 3.8). Given that eggs in the damage zone do not survive (Figure 3.5), it is not surprising that the forced fruit, with their smaller damage zones, had higher larval survival than natural fruit (Figure 3.7). Because of this experimental bias I consider the high and highly density-dependent mortality rates observed by Addicott and Bao (1999) and in my natural fruit (empty circles, Figure 3.7) to be accurate.

To summarize my results, I confirm Addicott and Bao's (1999) finding that there is a significant amount of egg mortality in retained fruit and that this mortality is very density-dependent, with an average of only one larva surviving per locule. Even with seven or more ovipositions in a locule, fewer than two larvae survive per locule on average. This mortality takes place before larvae begin feeding, and Addicott (1986) has reported that one larva consumes an average of 20% of the seeds in a locule, and two larvae in a locule consume an average of 40% of the seeds. The level of density-dependent *T. altiplanella* egg mortality Addicott and Bao (1999) and I have observed in *Y. kanabensis* fruit appears to be sufficient to prevent a shift of this interaction from mutualism to parasitism of the plant, regardless of how many moths visit flowers.

Additionally, I show that eggs in the damage zone do not survive, and damage zone mortality appears to be sufficient to explain most, if not all, of the egg mortality in fruit.

Since egg mortality in fruit takes place before larvae begin feeding it directly limits the level of seed consumption in fruit. Since the mortality rate is strongly density-dependent, the level of seed consumption is regulated, with total seed consumption in fruit being a highly unlikely occurrence. Another effect of the density-dependence of egg mortality in fruit may be that it helps to regulate the moth population size. How does regulating moth population size affect the costs and benefits of yuccas? Too few moths would clearly result in pollen – limitation and reduced yucca reproductive success. Therefore, reduced moth mortality rates at lower moth densities will help to stabilize the interaction by increasing moth density. As moth density increases, the average number of eggs laid per flower increases; *T. altiplanella* do not avoid ovipositing in previously visited flowers (Wilson and Addicott 1998). Oviposition damage to ovules increases with oviposition number, only levelling off slightly with many ovipositions (Figure 2.13). Oviposition damage can create a large loss of seeds, with half or more of the ovules in a locule being destroyed (personal observation). By helping to regulate the adult moth population size, the density-dependent egg mortality in fruit limits the number of adult moths ovipositing in flowers, and reduces the number of ovules lost to oviposition damage.

Given the density-dependent egg mortality that takes place in fruit, how does a yucca's selective abscission of flowers with heavy egg loads affect its fitness? On average, reducing the number of eggs in fruit via selective abscission of heavily egg-laden flowers will not substantially reduce the number of larvae feeding in fruit. Egg mortality is density-dependent, and the number of ovipositions in a locule is a poor predictor of the number of larvae surviving to 13 days (adjusted $r^2 = 0.08$, n=1066 locules). Yuccas appear to selectively abscise flowers based on the level of damage done to their ovules during oviposition (Fuller 1990, Millar 2001, and Table 2.2). Therefore, retained fruit have fewer ovules lost to oviposition damage than the average flower. There is variation in the size of the damage zone for any given number of eggs laid in a locule and a larger damage zone for a given number of eggs is likely to result in fewer surviving larvae. Occasionally, by retaining flowers based on small damage zones,

yuccas will make suboptimal retention decisions: if two flowers with the same number of eggs have different numbers of damaged ovules, retention of the least damaged flower may result in lower seed production, if more larvae survive to feed on seeds (Figure 3.9). However, flowers with fewer damaged ovules on average have fewer eggs laid in them as well (Figure 3.2). In general, retaining flowers with fewer damaged ovules should not affect the number of larvae feeding on seeds and should reduce the number of ovules lost to oviposition damage.

When selective abscission by yuccas was first reported (Pellmyr and Huth 1994), it was proposed to be the primary mechanism that prevented overexploitation of yuccas by yucca moths, maintaining the mutualism. Addicott and Bao (1999) subsequently suggested that selective abscission could not be a universal regulating mechanism in the yucca – yucca moth mutualism, as some *Tegeticula* species do not damage ovules during oviposition, and their associated yuccas do not selectively abscise flowers with many eggs. Nonetheless, those yuccas also gain a net benefit from the interaction. Despite Addicott and Bao's (1999) caution, selective abscission is still seen as the source of stability in the yucca – yucca moth mutualism, preventing overexploitation of seeds by larvae (e.g. Bronstein 2001). My results clearly indicate that while selective abscission of flowers with many damaged ovules reduces the number of ovules lost to oviposition damage, strongly density-dependent egg mortality in fruit is sufficient to prevent overexploitation of *Y. kanabensis* by *T. altiplanella*.

The source of *T. altiplanella* egg mortality appears to be the damage zone created by the moths themselves during oviposition. Keeley *et al.* (1984) also observed unhatched eggs in the damage zone of *Y. schidigera* fruit, suggesting that damage zone mortality may be general to yucca moths who damage ovules during oviposition. Addicott and Bao (1999) reported density-dependent mortality of *T. superficiella* in *Y. kanabensis* fruit, and the source of this mortality is unknown. *Tegeticula superficiella* oviposit into the carpel wall, rather than directly into the locule, and do not damage ovules. Being in the carpel wall, *T. superficiella* eggs may be exposed to abiotic mortality factors (Addicott and Bao 1999). An interesting alternative hypothesis is that the yucca has some sort of induced defence in response to *T. superficiella*. Addicott (1986) reported high levels of egg mortality in fruit of seven yucca species other than *Y. kanabensis*.

Keeley *et. al.* (1986) also found a high rate of yucca moth (*T. maculata*) mortality in *Y. whipplei* fruit and found only 3% - 25% of seeds in fruit eaten. An extreme level of yucca moth mortality in fruit was reported by Bao and Addicott (1998). They found a polymorphism in *Y. baccata*; some yuccas consistently produce fruit with inviable ovules in the apical region of fruit, where *T. baccatella* moth eggs are laid. The eggs in those "cheater" morphs always perish. Perhaps yucca moth egg mortality in fruit, resulting from a variety of mortality sources, is a key mechanism that is general to the *Yucca* genus, maintaining yuccas' net benefits in their pollination – seed predation mutualism.

Although it is clear that *T. altiplanella* egg mortality in fruit occurs mainly in the damage zone, it is not clear what, specifically, is causing the mortality. The ovules in the damage zone are generally more mushy than the developing seeds, which may affect the eggs. The damage zone is likely created directly by mechanical damage during oviposition resulting in early ovule abortion. A less parsimonious, but an interesting possibility is that *Y. kanabensis* adaptively aborts ovules in regions of flowers where it detects eggs, perhaps by chemical cues, creating the so-called "damage zone" in which eggs die. In this scenario the plant trades off some ovules in early abortion for the payback of fewer seeds lost to larvae later on. Many plants produce secondary metabolites that serve as chemical defences. Perhaps *Y. kanabensis* releases a chemical in the local region of perceived attack that kills eggs in that region. Several plants have recently been shown to have induced chemical defences specifically in response to eggs laid on or in them by herbivorous insects (Agrawal 2000). Whether the density-dependent egg mortality results from a coevolutionary adaptation of the yucca or is serendipitous is an interesting problem that remains to be solved. I predict that yuccas' ovule abortion and creation the damage zone is not an adaptive defence against larval feeding because yuccas that had more resources available per fruit (those with "forced" fruit) had smaller damage zones than yuccas with fewer resources available per fruit (those with full inflorescences) (Figure 3.8). It seems most likely that yuccas with limited resources to allocate to developing fruit cut off resources to ovules with varying levels of damage according to the level of resources available.

3.4 Conclusion

In summary, dissections of fruit from "natural" inflorescences that were able to selectively retain fruit confirmed Addicott and Bao's (1999) findings of highly density-dependent *Tegeticula altiplanella* egg mortality. On average, only one larva survived per locule. This level of mortality is clearly sufficient to ensure *Yucca kanabensis*' successful seed production, preventing *T. altiplanella* from becoming parasitic. Egg mortality is sufficient to explain the low levels of seed consumption observed in *Y. kanabensis*.

Given the high level of egg mortality that occurs in fruit, the role of selective abscission in regulating the costs incurred by yuccas becomes diminished. Selectively abscising flowers with many eggs does not significantly reduce the number of larvae surviving to feed on seeds; the number of ovipositions in a fruit is a poor predictor of the number of larvae that will feed in that fruit (adjusted $r^2 = 0.08$). Thus, selective abscission reduces the number of seeds lost to oviposition damage, but not the number of seeds lost to larval feeding.

Keeley *et. al.* (1986) report high levels of *T. maculata* egg mortality in *Y. whipplei* fruit and Addicott and Bao (1999) report density-dependent *T. superficiella* egg mortality in *Y. kanabensis* fruit. Further investigation will reveal whether yucca moth mortality in yucca fruit is a generalized factor central in maintaining all yucca – yucca moth mutualisms. It also remains to be determined whether egg mortality in fruit is caused by an adaptation of yuccas resulting from coevolution with the moth or a serendipitous "preadaptation" that facilitates the mutualism.

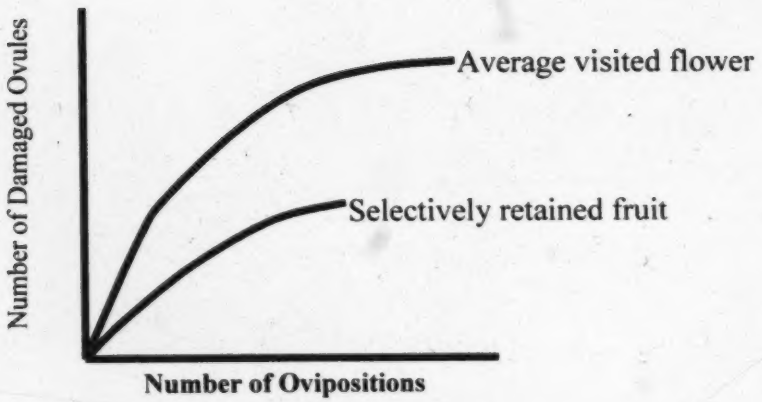

Figure 3.1. Graph to illustrate hypothetical selective abscission bias. Since yuccas selectively abscise flowers based on damage zone size, the relationship between damage zone and oviposition number in a flower is likely to differ between the average visited flower and the average selectively retained fruit. The lower line is shorter because selectively retained fruit will not have the high oviposition numbers observed in visited flowers.

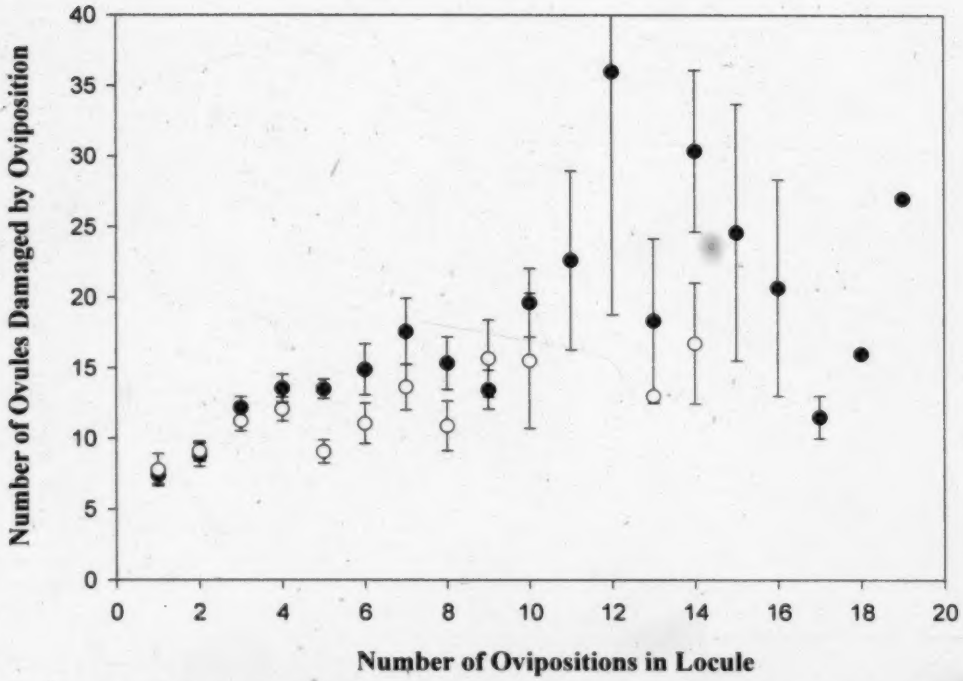

Figure 3.2. Selective abscission bias in experimental fruit. Black circles represent four day old flowers. White circles represent 14 day old fruit. Lines represent 1 standard error. The small amount of abscission that took place despite 90% bud removal created a small but statistically significant reduction in number of damaged ovules per oviposition in fruit (ANCOVA: F=5.62, df=1, n= 694, p=.018) and a lower maximum number of ovipositions in fruit as compared to flowers. This bias is not sufficient to explain the strong pattern observed in Figure 3.5.

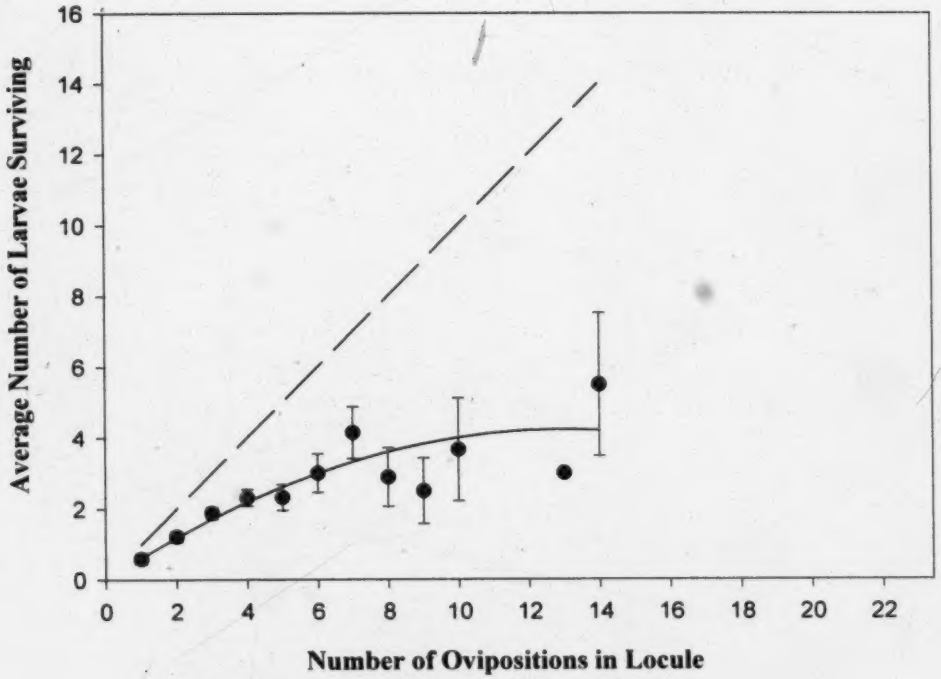

Figure 3.3. The number of surviving larvae in locules with varying numbers of ovipositions. Error bars represent 1 SE. Dashed line represents 1:1 relationship between ovipositions and larvae, for comparison. Solid line represents quadratic regression on data ($y=0.651x - 0.025x^2$, df=323, F=251.26, $p<0.001$, adjusted $r^2=0.61$, each term $p<0.001$).

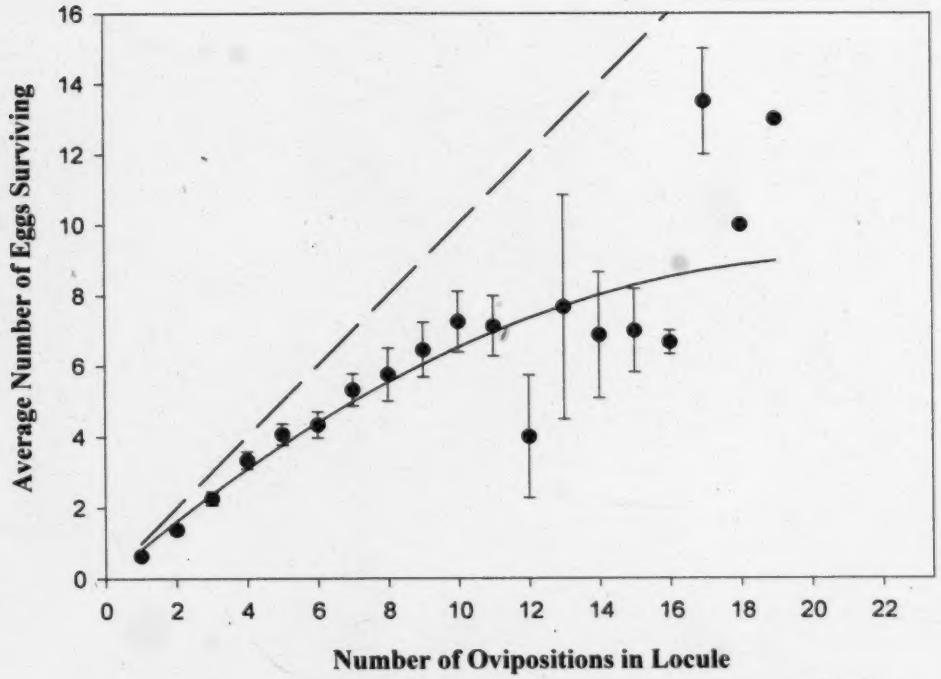

Figure.3.4. Number of eggs surviving to 4 days as a function of the number of ovipositions in a locule. Error bars represent 1 SE. Dashed line represents 1:1 relationship between ovipositions and eggs. Solid line represents quadratic regression ($y=0.853x-0.020x^2$, df=367, F=862.014, $p<0.001$, adjusted $r^2=0.82$, $p<0.001$ for both linear and quadratic terms)

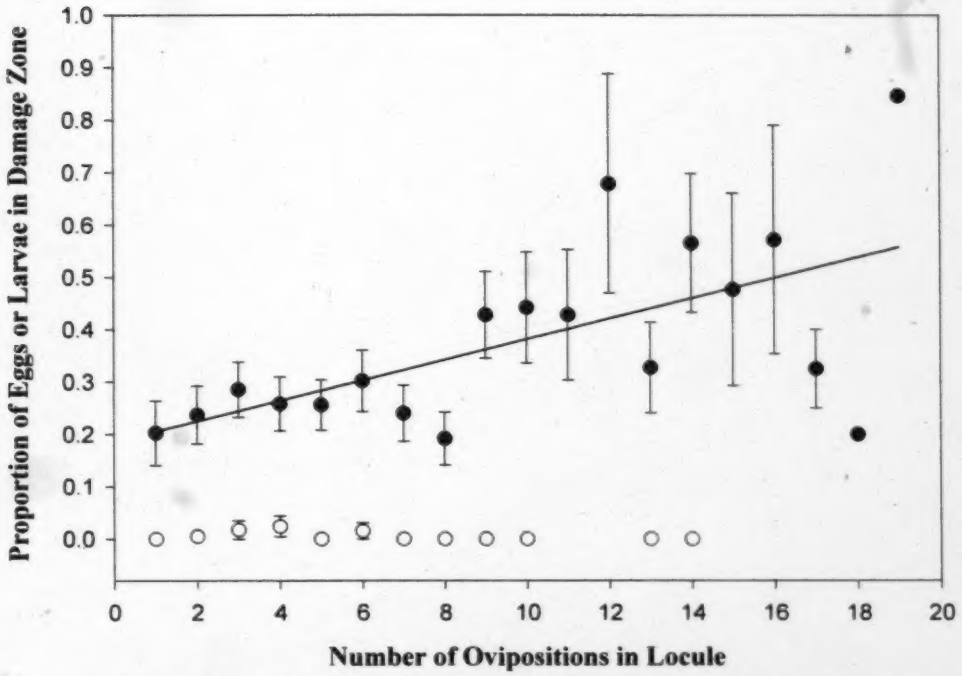

Figure 3.5. The proportion of eggs or larvae in the damage zone of a locule as a function of the number of ovipositions in the locule. Black circles represent averages for 4 day old locules, and white circles represent averages for 14 day old locules. Error bars represent 1 SE. Regression line for 4 day old flowers has significant linear term, indicating density dependence ($y=0.186+0.0196x$, $F=16.21$, $n=369$, $d.f=1$, $p<0.001$). Regression for 14 day old locules not significant, indicating that the proportion of larvae in the damage zone is not affected by the number of ovipositions: $F=.155$, $n=244$, $d.f=1$, $p=.694$. ANCOVA, 4d vs. 14d: $F=138.0$, $n= 560$, $d.f=1$, $p<0.001$.

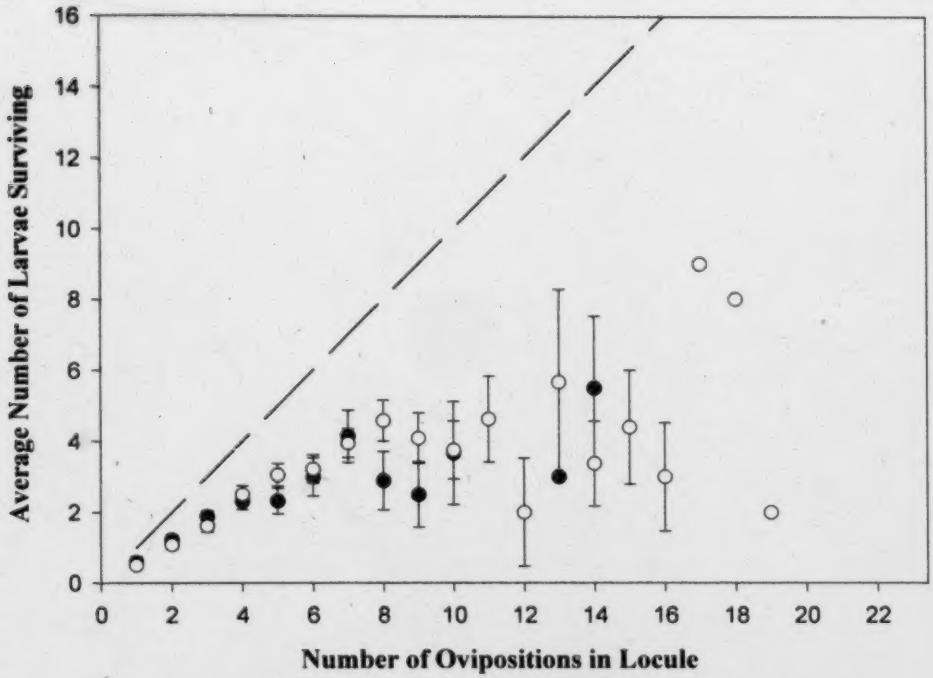

Figure 3.6. Predicted larval survival vs. observed larval survival. Number of larvae surviving to 14 days (black circles), and number of eggs predicted to survive to 14 days based on damage zone hypothesis (white circles) are not significantly different (F=.098, d.f=1, p=.754). Error bar represents 1 SE. The number of eggs predicted to survive to larval stage was the number of eggs in four day old locules that were outside of the damage zone. ANCOVA, predicted vs. observed: F=.098, d.f=1, p=.754

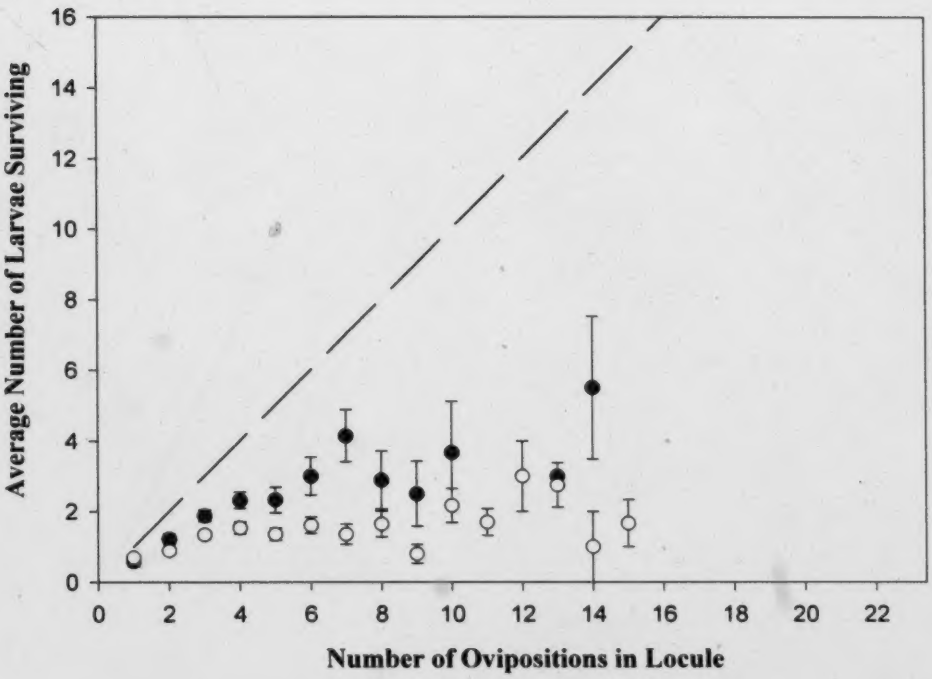

Figure 3.7. Larval survival in this experiment ("forced fruit") vs. selective abscission experiment ("natural fruit"). Black circles represent locules from this experiment and white circles represent locules from "selective abscission experiment". ANCOVA shows a significant difference in larval survival between fruit from the two experiments ($F=73.128$, d.f=1, n= 1391, $p<0.001$).

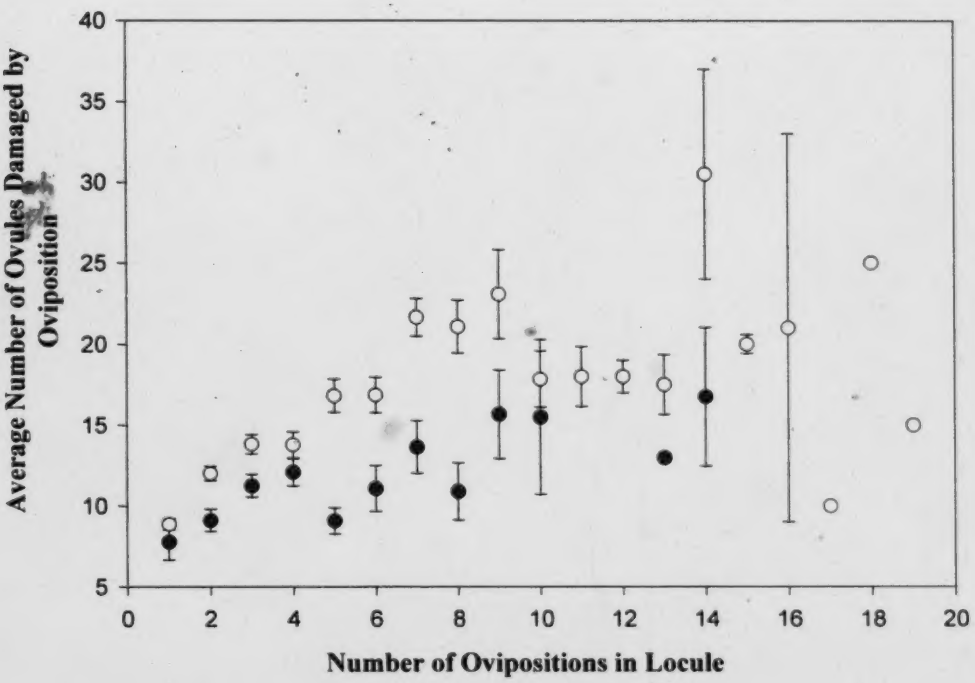

Figure 3.8. The number of damaged ovules is higher in "natural" fruit from selective abscission experiment (white circles) than in "forced" fruit from deep mortality experiment (black circles) ($F=42.381$, $d.f=1$, $n=1391$, $p<0.001$). Lines represent standard error.

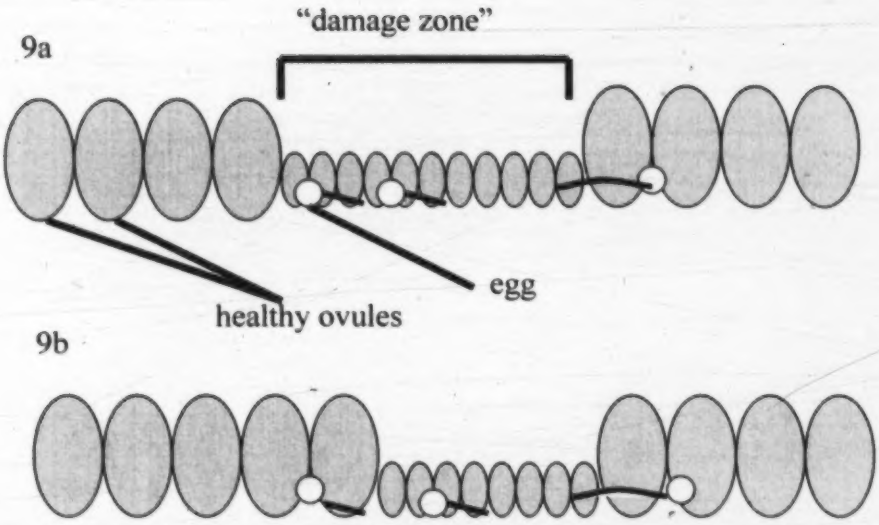

Figure 3.9 Schematic of hypothetical locules a and b. (Note that the total number of ovules in a real locule is approximately 65). Locule"a" has a larger damage zone than locule" b", and so it would be less likely to be retained by a yucca. However, the final seed production would be higher in locule "a" because only one of the eggs is outside the damage zone and likely to survive, whereas in locule" b" two eggs are outside the damage zone and so the feeding damage will likely be double.

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Chapter 4

Discussion

4.0 Conflicts of Interest in Mutualism

In the obligate pollination – seed predation mutualism between yuccas (*Yucca* spp., Agavaceae) and yucca moths (*Tegeticula* spp., Prodoxidae), moth larvae feed on developing seeds in yucca fruit, killing the seeds. Although yuccas and yucca moths depend on one another for sexual reproduction, there exists a conflict of interests because the primary cost incurred by yuccas is seed predation, and the primary benefit to yucca moths is seeds eaten. If yucca moths consumed the entire yucca seed crop, the negative effect on yucca population size would be in the distant future relative to moth generation time, since yuccas are long-lived and reproduce clonally as well as from seed. Thus, there would be a reduction in host availability in the long term, but this could not select for reduced exploitation on the part of moths. Some other more immediate trade-off or constraint must occur to explain the low seed predation rate observed in yucca fruit (15% on average, Addicott 1986).

Many mutualisms show conditionality, with gross costs and benefits varying with abiotic and biotic conditions, including partner density (Bronstein 1994). The benefits that yuccas gain from moths are minimal when moths are sparse and fruit production is pollinator-limited, and benefits increase with moth density until the pollination benefits are saturated (i.e. all ovules are fertilized). Although the yuccas' benefits saturate at high moth densities, the number of eggs laid per flower continues to increase with moth density, and thus the cost of seed predation may also continue to rise. The net benefit that yuccas gain from their mutualist could potentially decline dramatically at high moth densities if there were no mechanisms in place to regulate moth density or to limit seed predation.

In this thesis I examined the effects of two mechanisms on the regulation of moth density and seed consumption in the mutualism between *Tegeticula altiplanella* and *Yucca kanabensis*: selective flower abscission and egg mortality in fruit.

4.1 Selective Abscission Egg Mortality

In Chapter 2 I addressed the question of whether *T. altiplanella* egg mortality resulting from selective abscission was density-dependent, and thus potentially population regulating. Selective abscission of flowers with high egg loads was predicted to occur by Addicott (1986) and by Bull and Rice (1991), and was later confirmed in three yucca species (Pellmyr and Huth 1994, Richter and Weis 1995, Addicott and Bao 1999). However, selective abscission is not universal to the yucca – yucca moth interaction (Addicott and Bao 1999). For instance, *Y. kanabensis* also interacts with another yucca moth, *T. superficiella*, and does not selectively abscise flowers based on *T. superficiella* egg number. Therefore, any regulatory effects selective abscission might have only apply to those yucca – yucca moth interactions in which selective abscission takes place. Yuccas are generally resource-limited and can only retain approximately 10% of their flowers (Addicott 1986). *Yucca kanabensis* selectively abscises flowers with large numbers of *T. altiplanella* eggs (Addicott and Bao 1999). Eggs in abscised flowers invariably perish.

Egg mortality in abscised flowers is usefully partitioned into two categories: resource-limited abscission mortality and selective abscission mortality. Resource-limited abscission is the abscission that takes place when more flowers are visited than the yucca has resources to retain. When 10% of the flowers have received pollen, there is no resource-limited abscission, and thus no resource-limited abscission egg mortality. As visitation increases to 100%, resource-limited abscission mortality increases to 90%. However, once visitation reaches 100%, resource-limited abscission mortality ceases to be density-dependent; if yuccas randomly retain 10% of flowers, on average 90% of eggs will die in abscised flowers (Section 2.0). In other words, resource-limited abscission is a major source of egg mortality, but it ceases to be density-dependent at very high moth densities (such as at sites that I studied, with 90-100% visitation) and can not place an upper limit on moth densities.

However, *Y. kanabensis* selectively abscises flowers with many eggs, creating an additional source of mortality: selective abscission mortality, the egg mortality that occurs above and beyond resource-limited abscission mortality. Egg mortality resulting

from selective abscission can occur in any inflorescence that is resource-limited, and so egg mortality in the abscised flowers of any inflorescence can be partitioned into mortality due to resource-limited abscission and mortality due to selective abscission. In Chapter 2 I asked if selective abscission egg mortality was density-dependent. If it is, then at sites with medium moth density, egg mortality in abscised flowers will be strongly density-dependent, with both resource-limited and selective abscission mortality being density-dependent. At sites with high moth density, resource-limited abscission will create high but density-independent mortality, and selective abscission may help to regulate moth density.

The egg mortality that occurs due to selective abscission was not density-dependent in any of my sites during either of the seasons I measured it (Figure 2.8). There appear to be at least two reasons for density-independent mortality. The first is related to the distribution of eggs on flowers. Inflorescences that experienced higher moth densities had higher mean egg loads per flower, and the number of eggs in their least-visited flowers also increased. An inflorescence is receptive to moths for a period of approximately two weeks (personal observation) and adult moths typically live fewer than five days (Riley 1892, Dodd and Linhart 1994). Therefore, different flowers on an inflorescence may experience different moth densities. Some flowers on each yucca might experience a temporal refuge during which there would be sufficient moths to achieve pollination but with very few ovipositions. In this scenario, even as the peak moth density experienced by the yucca increased and the average egg load per flower increased accordingly, there would always be a few flowers with sufficient pollen and minimal egg numbers. If this occurred, the egg mortality resulting from selective abscission could be density-dependent. At my sites, during these years, this was not the case. The minimum number of eggs in yuccas flowers increased with the mean number of eggs in their flowers and the egg mortality due to selective abscission was density-independent.

Even yuccas that had higher variation in their egg loads and could potentially kill moths at a higher rate via selective abscission did not. The actual k value for selective abscission of a yucca was not correlated with its maximum potential k value (based on whether it retained exactly the flowers with the fewest eggs) (Figure 2.10). It is not clear

what was limiting selective abscission mortality. I tested the hypotheses that (1) yuccas use oviposition damage to ovules as a cue for selective abscission, and that (2) the level of damage tapers off with many ovipositions, preventing yuccas from distinguishing between flowers once they receive a threshold number of eggs. If yuccas could not distinguish from one another flowers with more than a threshold number of ovipositions, this would limit their ability to achieve density-dependent selective abscission. As a higher and higher proportion of flowers on an inflorescence had an egg load greater than the threshold, the selective capacity of the yucca would decline. Yuccas do appear to use oviposition damage as a cue for selective abscission (Section 2.2.2, Millar 2001, Fuller 1990). As I expected, oviposition damage did not increase linearly with oviposition number, but damage did not level off in the observed range (Figure 2.13). Flowers with more ovipositions have larger damage zones, on average. The imperfection of the damage zone cue, then, should not affect the density-dependence of selective abscission.

Addicott (1998) observed that yucca moth populations near Kanab appear to be relatively stable from year to year. My results suggest that selective abscission is unlikely to regulate moth population density and explain this apparent stability.

4.2 Egg Mortality in Retained Fruit

In contrast, I found that there was strong density-dependent within locule egg mortality in retained, developing fruit, with an overall average of approximately one larva surviving per locule (white circles, Figure 3.7). The mortality was so density-dependent that the number of eggs laid in a locule was a very poor predictor of the number of surviving larvae (adjusted r^2=0.08, d.f.=1063). The egg mortality that took place between day four and day 14 was in the "damage zones" of locules (Figure 2.5); Eggs inside the damage zone of locules faced almost 100% mortality and eggs outside the damage zone largely survived to the larval stage (Figure 3.5). Damage zone egg mortality was sufficient to explain the mortality between day four and day 14; If all of the eggs in the damage zone of four day old locules perished and all of the eggs outside the damage zones survived to day 14, the survival rate would closely match the survival rate observed in 14 day old locules (Figure 3.6).

4.3 Conclusion: A New Perspective on Regulation of Yucca – Yucca Moth Mutualism and Future Research

These results indicate that, although selective abscission has been seen as the primary regulator of seed predation costs in yucca – yucca moth mutualisms (Pellmyr and Huth 1994, Bronstein 2001), it is actually *T. altiplanella* egg mortality in developing *Y. kanabensis* fruit that explains the low seed predation costs. Since the number of eggs laid is such a poor predictor of the number of larvae that will feed in a locule, selective abscission can have a limited effect on the number of larvae feeding on a yucca's seeds. However, selective abscission does reduce the number of oviposition-damaged ovules in retained fruit (Table 2.2). Flowers with fewer damaged ovules generally have fewer eggs in them (Figure 2.14), and so selectively retaining flowers with small damage zones should generally result in the same number of larvae feeding in the flower but fewer ovules lost to oviposition damage. This is also important for regulating yuccas' costs, as oviposition damage can be quite significant, with up to half of the ovules in a locule being damaged by oviposition (personal observation).

My results are interestingly consistent with studies of three other similar mutualisms in which insect mortality at the appropriate life stage helps to regulate the host's costs and maintain the mutualism. The senita cactus *(Lophocereus schotti Cactaceae)* is primarily pollinated by senita moths *(Upiga virescens* Pyralidae) in the Sonoran Desert (Fleming and Holland 1998). As well as pollinating senita cactus flowers, the senita moth oviposits an egg on the tip of a flower petal. If the larva survives, it crawls to the bottom of the closed corolla, chews through the ovary wall and eats developing seeds. Fruits that contain larvae are abscised and produce no viable seeds. However, Flemming and Holland found that there was very high egg mortality on flower petals, with only approximately 20% of eggs surviving to feed on cactus seeds. In total, only about 30% of senita moth pollinated fruits were destroyed by senita moths.

Second, another obligate pollination – seed predation mutualism occurs between the globeflower *Trollius europaeus* and *Chiastocheta* sp. flies (Anthomyiidae). The flies

are considered to be the primary pollinator of the globeflower, and the larvae feed only on the seeds of the plant. Jaeger and colleagues (2001) found that seed predation increased only weakly with egg load in flowers, ranging from a mean of 15% to 40%, depending on the population, and never exceeding 85%. In this case, the eggs do not face high mortality; 70 to 80% of eggs hatch into larvae. However, they found that interference competition between larvae in fruit resulted in a significant decline in number of seeds eaten per egg laid as number of eggs laid on a flower (Jaeger *et al.* 2001). It is not clear whether this interference competition resulted in density-dependent mortality of fly larvae (as in our case with *T. altiplanella* egg mortality), but it certainly limited the seed loss experienced by flowers. In addition to intraspecific competition of the fly helping to regulate globeflower seed predation costs, Jaeger and colleagues report a globeflower behaviour that appears to further reduce seed predation. There are approximately 30 carpels with 12 ovules in each in a globeflower and fly larvae can readily cross from carpel to carpel, potentially creating large levels of seed destruction. However, the fruit dehisce before the end of larval feeding activity, separating the carpels and preventing larvae from moving from one to the next.

Third, an ecological analogue to the pollination – seed predation mutualism is seen in *Botanophila* flies and the fungus, *Epichloë elymi* (Bultman *et al.* 1999). The fly's only oviposition site is on *Epichloë* and in a stereotypical behaviour following oviposition the flies defecate spermatia (gametes). The flies appear to be the primary or even only vector for *Epichloë* spermatia, so the mutualism is obligate for both parters' sexual reproduction. The fly larvae feed on the fungal ascospores (the fungus' ecological analogue to seeds, ascospores are a product of cross-fertilization and act as propagules by infecting new grass hosts). During three years of observations, Bultman and colleagues found that the benefits to the fungus (fertilization) outweighed the costs (feeding damage), and further found that the rates of feeding damage were regulated by high and density-dependent levels of fly egg/larval mortality of unknown origin.

A clear, complete picture of the yucca – yucca moth mutualism remains elusive. Do all yucca moths that create damage zones experience density-dependent egg mortality? What causes eggs in the damage zone to perish? Does the yucca have an anti-herbivore defence in response to detection of eggs or damaged ovules or do the moths

create their own progeny's mortality by piercing ovules during oviposition and thereby creating an unfavourable physical or chemical environment? Another yucca moth species, *T. superficiella*, also oviposits into *Y. kanabensis* flowers, but into the carpel wall instead of the locular cavity. Addicott and Bao (1999) report that these eggs also face high mortality levels in retained fruit, but the cause of their mortality remains unknown. Is there any relationship between the egg mortality experienced by *T. altiplanella* inside locules and the mortality of *T. superficiella* in the carpel wall? The impact of egg mortality on seed production depends on the relationship between number of larvae in a locule and level of seed damage, and whether this relationship is density-dependent. Two larvae feeding in a locule consume twice as many seeds as one larva does, but there are only limited data on the amount of larval feeding in locules with more than two larvae (Addicott 1986, James 1998). Is larval feeding in locules density-dependent and how does larval competition interact with egg mortality to regulate seed consumption? Since the rare fruit that do contain many surviving larvae do have all of their seeds consumed (Rau 1945, Addicott 1986), I predict that larval interactions will not be as important for limiting seed predation. In conclusion, this study reports that selective abscission cannot limit seed predation by *T. altiplanella* in *Y. kanabensis*, and that strongly density-dependent egg mortality in retained fruit does limit seed predation, preventing this mutualism from shifting to a parasitism of the yucca. Future research of other yuccas will reveal how universal density-dependent egg mortality is as a mechanism that limits seed predation by yucca moths.

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